

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

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21 **Abstract**

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

40

41 **Keywords**

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

44

45 **Introduction**

46

47 Synthetic cannabinoids (SCs) have been introduced as drugs of abuse over the past years as a
48 legal alternative to cannabis. They are mainly being sold mixed with herbal substances, but can
49 also be bought in resin-like material, as powder, and in liquid e-cigarette refills. The existence
50 of synthetic cannabinoid receptor agonists in the abuse market was first reported in 2009 by
51 Japanese and German researchers ^[1-3]. In the UK, the first generation of SCs were banned in
52 2009. The rise of new compounds has made it more and more difficult for toxicologists to keep
53 up to date with standard analytical techniques and consequently has put users at risk when
54 abusing these substances. In addition, users often take new substances unknowingly, because
55 branded products change their ingredients over time and, in particular, when new legislation is
56 put into place that bans existing SCs.

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

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

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

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

84

85 **Material and methods**

86

87 **Reagents and Chemicals**

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

95 **Samples**

96 Three periods in the sampling campaign can be distinguished: 1) December 1st, 2012 –
97 February 26th, 2013, the date when the new ban in the UK came into place, 49 samples were
98 bought just before the new legislation. 2) February 26th, 2013 – June 30th, 2013, 54 samples
99 were acquired immediately after the ban was in place. Samples from these first two sampling
100 campaigns were bought from websites and head-shops or acquired from police authorities. All
101 samples were powders or herbal material sold as smoking mixtures. 3) July 1st, 2013 – January
102 31st, 2015, 85 samples were bought from the Internet regardless of the description. Among
103 these samples were powders, herbal mixtures, one resin-like sample, and liquid e-cigarette
104 refills, which we subject to detailed analysis by LC–QTOF-MS(/MS).

105 **Sample preparation**

106 Approximately 1 mg of powder was dissolved in 1 mL of methanol in a 1.5 mL polypropylene
107 tube. Solutions were then vortexed for 1 min or subjected to ultrasonic-assisted extraction for
108 15 min, and afterwards centrifuged at 8,000 rpm (6,030 g) for 5 min. For herbal mixtures,
109 approximately 50 mg was mixed in 1 mL of methanol and vortexed for 30 min and centrifuged

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

113 **Instrumentation**

114 LC–QTOF-MS(/MS) analyses were performed using an Acquity Ultra-Performance Liquid
115 Chromatography UPLC system (Waters, Milford, MA, USA), which was interfaced to a hybrid
116 quadrupole-orthogonal acceleration-TOF mass spectrometer (QTOF XEVO G2, Waters
117 Micromass, Manchester, UK), using an orthogonal Z-spray-ESI interface operating in positive
118 ion mode. The chromatographic separation was performed using an Acquity UPLC BEH C18
119 analytical column (100 × 2.1 mm with 1.7 µm particle size; Waters). The column temperature
120 was set to 40 °C. The mobile phases used were A = H₂O and B = MeOH, both with 0.01%
121 HCOOH, at a flow rate of 300 µL/min [more details in supplementary material (SM) and ^[7]].

122 GC–MS analyses were done using an Agilent 7890A GC with 5975C VL MSD
123 (Agilent, Santa Clara, CA, USA) equipped with a split-splitless injector and an HP5-MS
124 column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) and running on
125 Agilent ChemStation. 1 µL was injected using 5:1 split ratio. The column was held at 80°C for
126 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
127 range of *m/z* 40 to 400 was scanned with scan-time 0.25 sec.

128 **Results and discussion**

129 **LC-QTOF-MS(/MS) analysis of synthetic cannabinoids**

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

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

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

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

159

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

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

166 In all four compounds identified, the most abundant product ion at 30 eV corresponded
167 to the adamantyl group (ion C, m/z 135.1174, C₁₀H₁₅) (Fig. 2a). Table 1 shows the product ions
168 as well as the corresponding elemental compositions for all cannabinoids included in this
169 group. Regarding SDB-006 (m/z 321.1967), the product ion resulting from the breaking of the
170 central amide (m/z 214.1232) and that corresponding to the pentyl indole group (m/z 188.1439)
171 are the most abundant ones (Fig. 2b; Table 1). LC-QTOF-MS(/MS) spectra at different
172 collision energies for all cannabinoids in this section are included in SM (Figs. S.1-S.5).

173

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

175 Four cannabinoids belong to this group of compounds containing a quinolinyl ester: PB-22,
176 5F-PB-22, BB-22 and 5F-NPB-22 (Table 2). In addition, two related compounds were also
177 identified and are discussed here. NM-2201 is structurally similar to these cannabinoids; the

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

183 For PB-22, 5F-PB-22, BB-22, 5F-NPB-22 and NM-2201, the main product ion (B) was
184 formed by cleavage of the ester bond (Table 2; Fig. 3). Another important product ion (E) was
185 related to presence of an indole or indazole in the structure. For indole-based structures, PB-
186 22, 5F-PB-22, BB-22 and NM-2201, ion E at m/z 144.0449 (C_9H_6NO) was observed, whereas
187 for indazole-based structures and as 5F-NPB-22 and 5F-MN-18, the product ions E
188 corresponded to m/z 145.0402 ($C_8H_5N_2O$). Additionally, for 5F-NPB-22 and 5F-MN-18, the
189 m/z 213.1028 (ion C) was observed, corresponding to the loss of hydrogen fluoride (HF) from
190 m/z 233 (Figs. S.6-S.11).

191

192 **Cannabinoids with a branched end group**

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

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

203

204 **Cannabinoids with a *para*-fluorotoluene chain**

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

212

213 **Cannabinoids with two chromatographic peaks**

214 Two chromatographic peaks were observed in the LC-QTOF-MS chromatograms at the
215 expected m/z for five compounds, concretely AB-FUBINACA, ADB-PINACA, AB-PINACA,
216 5F-AB-PINACA, and AB-CHMINACA (Table 3; Fig. 5a). For these compounds, the two
217 chromatographic peaks presented different fragmentation, being all compatible with the
218 structure of the corresponding cannabinoid. All of them possess a terminal amino group and an
219 enantiomeric carbon at the linker part. Moreover, some common product ions were also
220 observed, but with different relative intensities. In all cases, the first chromatographic peak
221 presented an abundant protonated molecule, whereas the second presented as peak base at 10
222 eV with the product ion corresponding to the loss of NH_3 . This did not happen in GC-MS,
223 where only one chromatographic peak was observed. This might be explained by the
224 occurrence of rotamers. However, isolation and further spectroscopic experiments is needed to
225 confirm and support this hypothesis.

226

227 **Cannabinoids with a carbonyl link**

228 THJ-018 and THJ-2201 have similar structures, differing only in the absence and presence of
229 a fluorine atom at the end of the chain, respectively. The main product ions were ion F, at m/z
230 145.0402 ($C_8H_5N_2O$), due to the carbonyl-indazole group, and ion B, which corresponded to
231 the loss of the naphthalene group ($C_{10}H_8$) (Table 5). In the case of THJ-2201 (Fig. 6a), a
232 subsequent loss of HF was also observed (ion C). (Figs. S.22-S.23).

233 Other two cannabinoids were included in the same group, EG-018 (Fig. 6b) and BZ-
234 2201. They present similar fragmentation (Table 5), with the major product ions being m/z
235 155.0497 ($C_{11}H_7O$, corresponding to the carbonyl-naphthalene group) and 127.0548
236 (corresponding to the naphthalene group) (Figs S.24-S.25).

237

238 **Unidentified novel synthetic cannabinoids**

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

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

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

252

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

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

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

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

269 Up to January 2015 (sampling period 3), a further 21 SCs, not previously reported in
270 the UK market, were identified: 5F-Cumyl-PINACA, FUB-PB-22, 5F-NPB-22, EG-018, THJ-
271 018, THJ-2201, NM-2201, BZ-2201, F2201, SDB-006, dealkyl-SDB-006, 5F-MN-18, APICA,
272 MDMB-CHMICA (also incorrectly known as MMB-CHMINACA), AB-CHMINACA, AB-
273 PINACA, 5F-AB-PINACA, ADB-PINACA, 5F-AMB, AB-FUBINACA and ADB-
274 FUBINACA. As it can be seen, some of these compounds were structurally-related to earlier
275 found cannabinoids. For example, in 5F-Cumyl-PINACA, the adamantyl group of 5F-
276 APINACA is replaced by a cumyl group. FUB-PB-22 replaces the fluoropentyl chain of 5F-

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

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

290

291 **Sampling period immediately before and after the ban**

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

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

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

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

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

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

337 After the ban, four brands were sampled more than once and two of them did not change
338 their ingredients, while two did. “Clockwork Orange” and “Chillem Blue” always contained
339 5F-AKB48 as the only active ingredient, while “Dutchy” contained either 5F-AKB48 or
340 AKB48 and “Magic Dragon” contained either 5F-AKB48 or 5F-PB-22. Hence, it seems that it
341 was not only due to the ban that manufacturers decided to switch to other ingredients.

342

343 **New physical forms for synthetic cannabinoids**

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

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

355

356

357 **Conclusions**

358 In this work, we have surveyed the UK legal high market between December 2012 and January
359 2015. Our results reveal that the legislative ban succeeded in pushing the corresponding
360 compounds from the UK market, but only one of the banned compounds (UR-144) detected
361 after the date when the ban came into place (February 26th, 2013). However, a risk of banning
362 existing compounds is the emergence of new compounds (which as our result show, did
363 happen), with unknown and potentially more dangerous effects. Another problem associated
364 with banning compounds is the lack of information for both drug users and healthcare workers.
365 Drug users do not know what they are taking after a ban, because branded products change
366 ingredients or vendors mislabel products to be able to sell leftover stock. For healthcare and
367 forensic professionals, there is little knowledge on new compounds, and they might be missed
368 in drug screenings.

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

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

384

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396

397 **Compliance with ethical standards**

398

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

400

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

403

404

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446 **TABLES**

447 **Table 1** Product ions obtained by liquid chromatography – quadrupole time-of-flight- tandem mass spectrometry (LC–QTOF-MS/MS) for
 448 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 ₂ O	214.1232 C ₁₄ H ₁₆ NO	135.1174 C ₁₀ H ₁₅	107.0861 C ₈ H ₁₁	93.0704 C ₇ H ₉	79.0548 C ₆ H ₇	14.9
5F-APICA (STS-135)	383.2499 C ₂₄ H ₃₂ N ₂ OF	232.1138 C ₁₄ H ₁₅ NOF	135.1174 C ₁₀ H ₁₅	107.0861 C ₈ H ₁₁	93.0704 C ₇ H ₉	79.0548 C ₆ H ₇	14.1
APINACA (AKB48)	366.2545 C ₂₃ H ₃₂ N ₃ O		135.1174 C ₁₀ H ₁₅	107.0861 C ₈ H ₁₁	93.0704 C ₇ H ₉	79.0548 C ₆ H ₇	15.5
5F-APINACA (5F- AKB48)	384.2451 C ₂₃ H ₃₁ N ₃ OF		135.1174 C ₁₀ H ₁₅	107.0861 C ₈ H ₁₁	93.0704 C ₇ H ₉	79.0548 C ₆ H ₇	14.5
SDB-006	321.1967 C ₂₁ H ₂₅ N ₂ O	214.1232 C ₁₄ H ₁₆ NO	188.1439 C ₁₃ H ₁₈ N	144.0449 C ₉ H ₆ NO	132.0813 C ₉ H ₁₀ N	91.0548 C ₇ H ₇	12.9

449 *RT* retention time in minutes

450

451 **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	214.1232			144.0449	116.0500		13.7
	C ₂₃ H ₂₃ N ₂ O ₂	C ₁₄ H ₁₆ NO			C ₉ H ₆ NO	C ₈ H ₆ N		
5F-PB-22	377.1665	232.1138			144.0449	116.0500		12.5
	C ₂₃ H ₂₂ N ₂ O ₂ F	C ₁₄ H ₁₅ NOF			C ₉ H ₆ N	C ₈ H ₆ N		
BB-22	385.1916	240.1388			144.0449	116.0500		14.2
	C ₂₅ H ₂₅ N ₂ O ₂	C ₁₆ H ₁₈ NO			C ₉ H ₆ NO	C ₈ H ₆ N		
NM-2201	376.1711	232.1138			144.0449	116.0500		14.1
	C ₂₄ H ₂₃ NO ₂ F	C ₁₄ H ₁₅ NOF			C ₉ H ₆ NO	C ₈ H ₆ N		
5F-NPB-22	378.1618	233.1090	213.1028	177.0453	145.0402	117.0453	90.0344	11.9
	C ₂₂ H ₂₁ N ₃ O ₂ F	C ₁₃ H ₁₄ N ₂ OF	C ₁₃ H ₁₃ N ₂ O	C ₁₂ H ₅ N ₂	C ₈ H ₅ N ₂ O	C ₇ H ₅ N ₂	C ₆ H ₄ N	
5F-MN-18	376.1825	233.1090	213.1028	177.0453	145.0402	117.0453	90.0344	13.4
	C ₂₃ H ₂₂ N ₃ OF	C ₁₃ H ₁₄ N ₂ OF	C ₁₃ H ₁₃ N ₂ O	C ₁₂ H ₅ N ₂	C ₈ H ₅ N ₂ O	C ₇ H ₅ N ₂	C ₆ H ₄ N	

452

453

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 ₄ O ₂	328.2025 C ₁₉ H ₂₆ N ₃ O ₂	300.2076 C ₁₈ H ₂₆ N ₃ O	215.1184 C ₁₃ H ₁₅ N ₂ O			145.0398 C ₈ H ₅ N ₂ O	13.0
AB-PINACA ^a	331.2134 C ₁₈ H ₂₇ N ₄ O ₂	314.1869 C ₁₈ H ₂₄ N ₃ O ₂	286.1919 C ₁₇ H ₂₄ N ₃ O	215.1184 C ₁₃ H ₁₅ N ₂ O			145.0398 C ₈ H ₅ N ₂ O	12.2
5F-AB-PINACA ^a	349.2040 C ₁₈ H ₂₆ N ₄ O ₂ F	332.1774 C ₁₈ H ₂₃ N ₃ O ₂ F	304.1825 C ₁₇ H ₂₃ N ₃ OF	233.1090 C ₁₃ H ₁₄ N ₂ OF	213.1028 C ₁₃ H ₁₃ N ₂ O	177.0463 C ₁₂ H ₅ N ₂	145.0398 C ₈ H ₅ N ₂ O	11.0
5F-Cumyl-PINACA	368.2138 C ₂₂ H ₂₇ N ₃ OF			233.1090 C ₁₃ H ₁₄ N ₂ OF	213.1028 C ₁₃ H ₁₃ N ₂ O	177.0463 C ₁₂ H ₅ N ₂	145.0398 C ₈ H ₅ N ₂ O	13.0
AB-CHMINACA ^a	357.2291 C ₂₀ H ₂₉ N ₄ O ₂	340.2025 C ₂₀ H ₂₆ N ₃ O ₂	312.2076 C ₁₉ H ₂₆ N ₃ O	241.1341 C ₁₅ H ₁₇ N ₂ O			145.0398 C ₈ H ₅ N ₂ O	13.1
MDMB-CHMICA	385.2491 C ₂₃ H ₃₃ N ₂ O ₃			240.1388 C ₁₆ H ₁₈ NO			144.0441 C ₉ H ₆ NO	14.1
5F-AMB	364.2036 C ₁₉ H ₂₇ N ₃ O ₃ F	332.1774 C ₁₈ H ₂₃ N ₃ O ₂ F	304.1825 C ₁₇ H ₂₃ N ₃ OF	233.1090 C ₁₃ H ₁₄ N ₂ OF	213.1028 C ₁₃ H ₁₃ N ₂ O	177.0463 C ₁₂ H ₅ N ₂	145.0398 C ₈ H ₅ N ₂ O	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)

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

Compound	A=[M+H]⁺	B	C	D	E	RT
AB-FUBINACA ^a	369.1727 C ₂₀ H ₂₂ N ₄ O ₂ F	352.1461 C ₂₀ H ₁₉ N ₃ O ₂ F	324.1512 C ₁₉ H ₁₉ N ₃ OF	253.0777 C ₁₅ H ₁₀ N ₂ OF	109.0454 C ₇ H ₆ F	11.5
ADB-FUBINACA	383.1883 C ₂₁ H ₂₄ N ₄ O ₂ F	366.1618 C ₂₁ H ₂₁ N ₃ O ₂ F	338.1669 C ₂₀ H ₂₁ N ₃ OF	253.0777 C ₁₅ H ₁₀ N ₂ OF	109.0454 C ₇ H ₆ F	11.9
FUB-PB-22	397.1352 C ₂₅ H ₁₈ N ₂ O ₂ F			252.0825 C ₁₆ H ₁₁ NOF	109.0454 C ₇ H ₆ F	12.9

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

462

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 ₂ O	215.1184 C ₁₃ H ₁₅ N ₂ O				145.0402 C ₈ H ₅ N ₂ O	127.0548 C ₁₀ H ₇	117.0453 C ₇ H ₅ N ₂	90.0344 C ₆ H ₄ N	14.7
THJ-2201	361.1716 C ₂₃ H ₂₂ N ₂ OF	233.1090 C ₁₃ H ₁₄ N ₂ OF	213.1028 C ₁₃ H ₁₃ N ₂ O		177.0453 C ₁₂ H ₅ N ₂	145.0402 C ₈ H ₅ N ₂ O	127.0548 C ₁₀ H ₇	117.0453 C ₇ H ₅ N ₂	90.0344 C ₆ H ₄ N	13.6
BZ-2201	361.1716 C ₂₃ H ₂₂ N ₂ OF	233.1090 C ₁₃ H ₁₄ N ₂ OF		155.0497 C ₁₁ H ₇ O	177.0453 C ₁₂ H ₅ N ₂	145.0402 C ₈ H ₅ N ₂ O	127.0548 C ₁₀ H ₇	117.0453 C ₇ H ₅ N ₂	90.0344 C ₆ H ₄ N	13.2
EG-018	392.2014 C ₂₈ H ₂₆ NO			155.0497 C ₁₁ H ₇ O		145.0402 C ₈ H ₅ N ₂ O	127.0548 C ₁₀ H ₇			15.8

464

466 **FIGURE CAPTIONS**

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

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

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

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

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

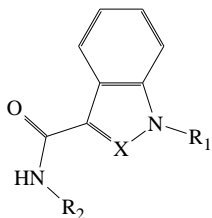
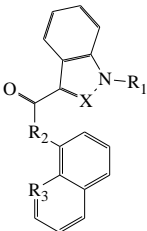
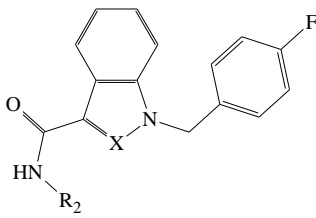
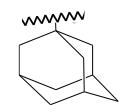
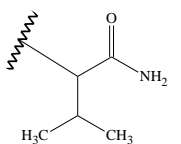
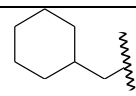
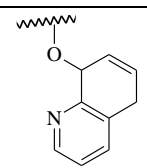
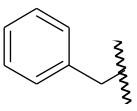
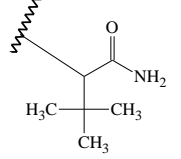
480 **Fig. 6** LC–QTOF-MS/MS spectra of **a** THJ-2201 and **b** EG-018, at different collision energies
481 with product ions identified together with the probable fragmentation modes

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

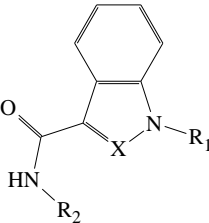
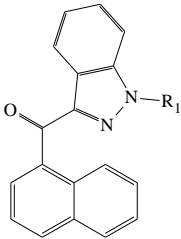
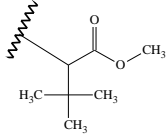
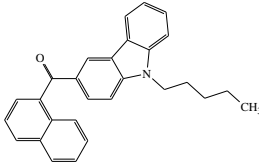
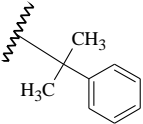
484 **Fig. 8** LC–QTOF-MS/MS spectra at different collision energies for unidentified compound **2**

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

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

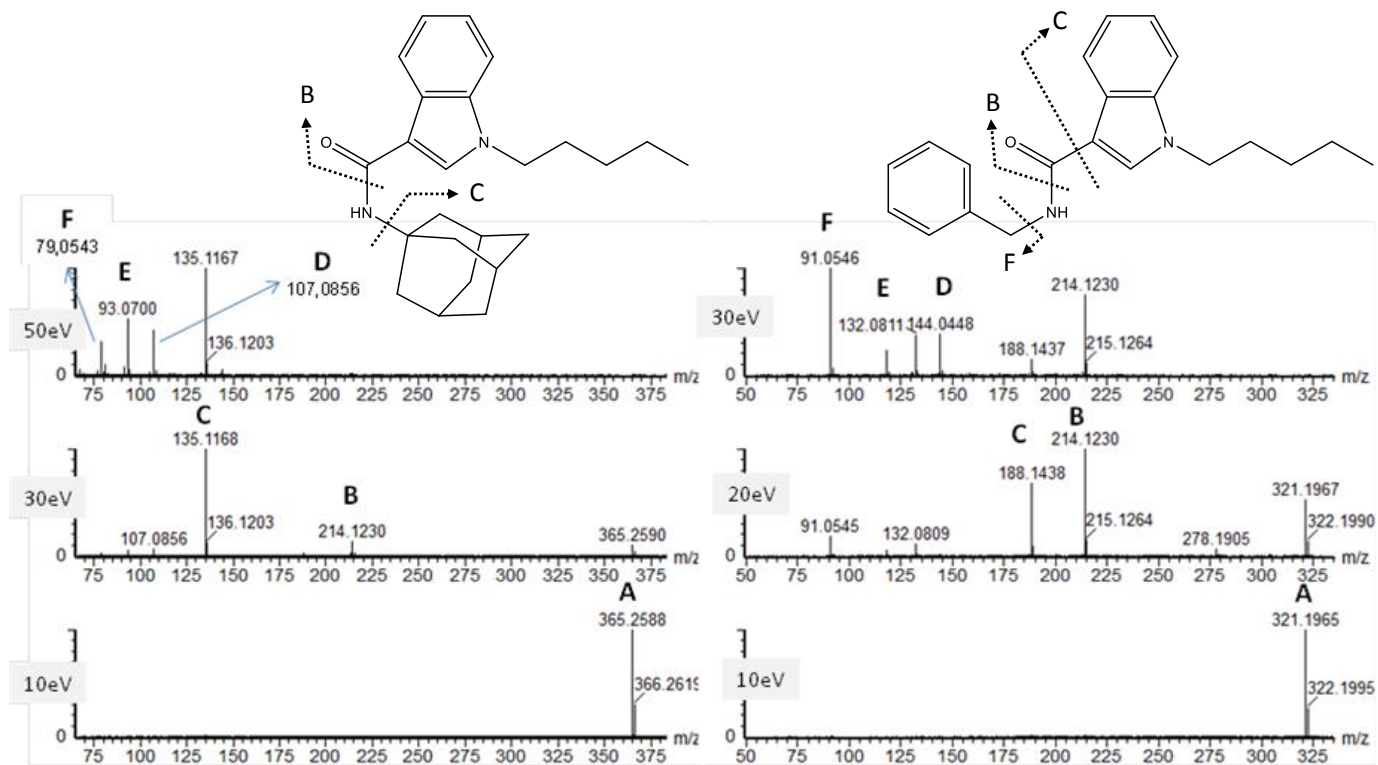
SC with adamantyl amide group				SC with quinolyl esters					SC with a para-fluorotoluene chain		
											
Compound	X	R ₁	R ₂	Compound	X	R ₁	R ₂	R ₃	Compound	X	R ₂
APICA (2NE1)	C	Pentyl	 Adamantyl	PB-22	C	Pentyl	O	N	AB-FUBINACA	N	 1-(Aminocarbonyl)-2- methylpropyl
5F-APICA (STS-135)	C	5-Fluoropentyl	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-Quinololinol, 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			

489 **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

490 **Fig. 1 (continue)**

491



a

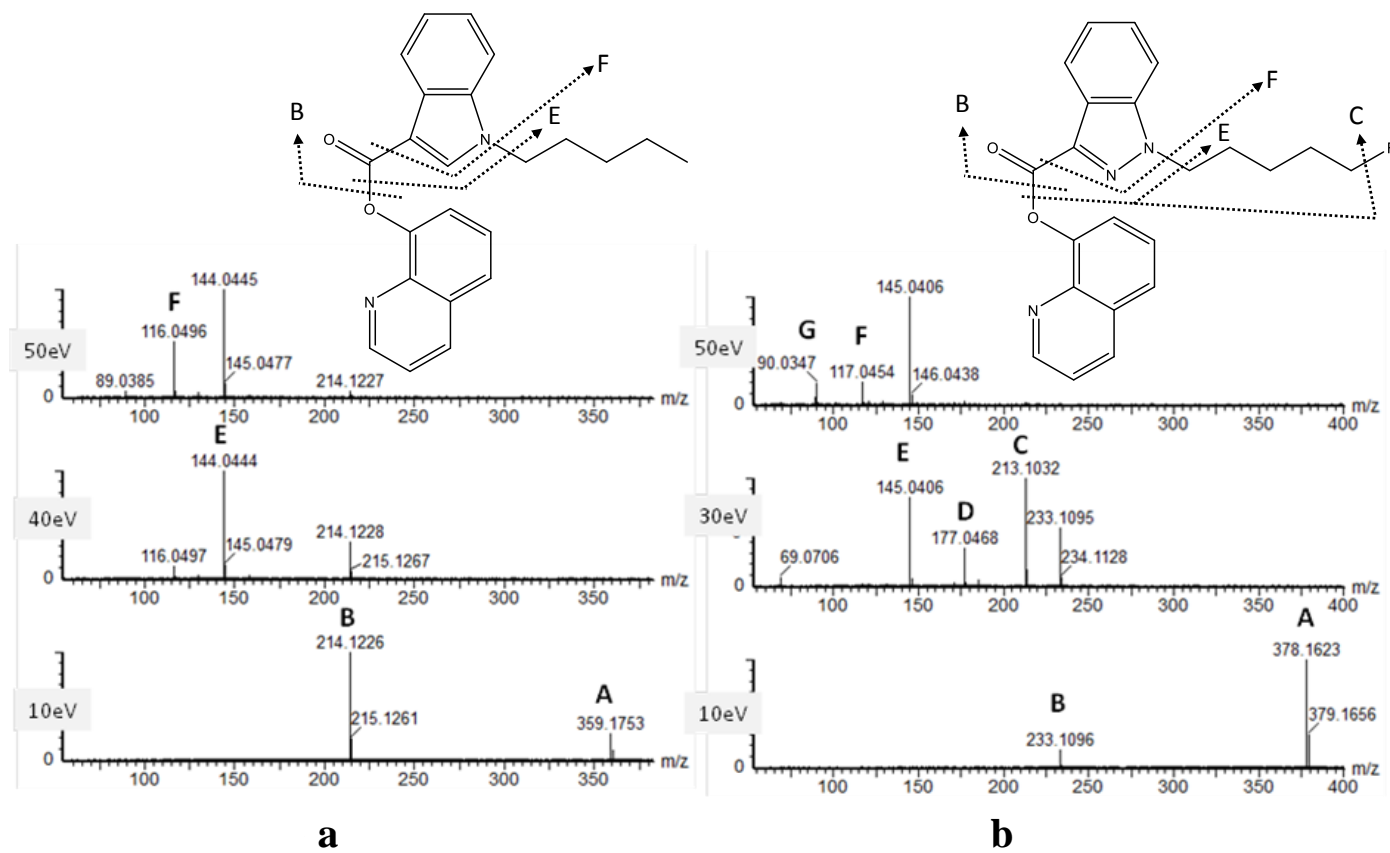
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493 **Fig. 2**

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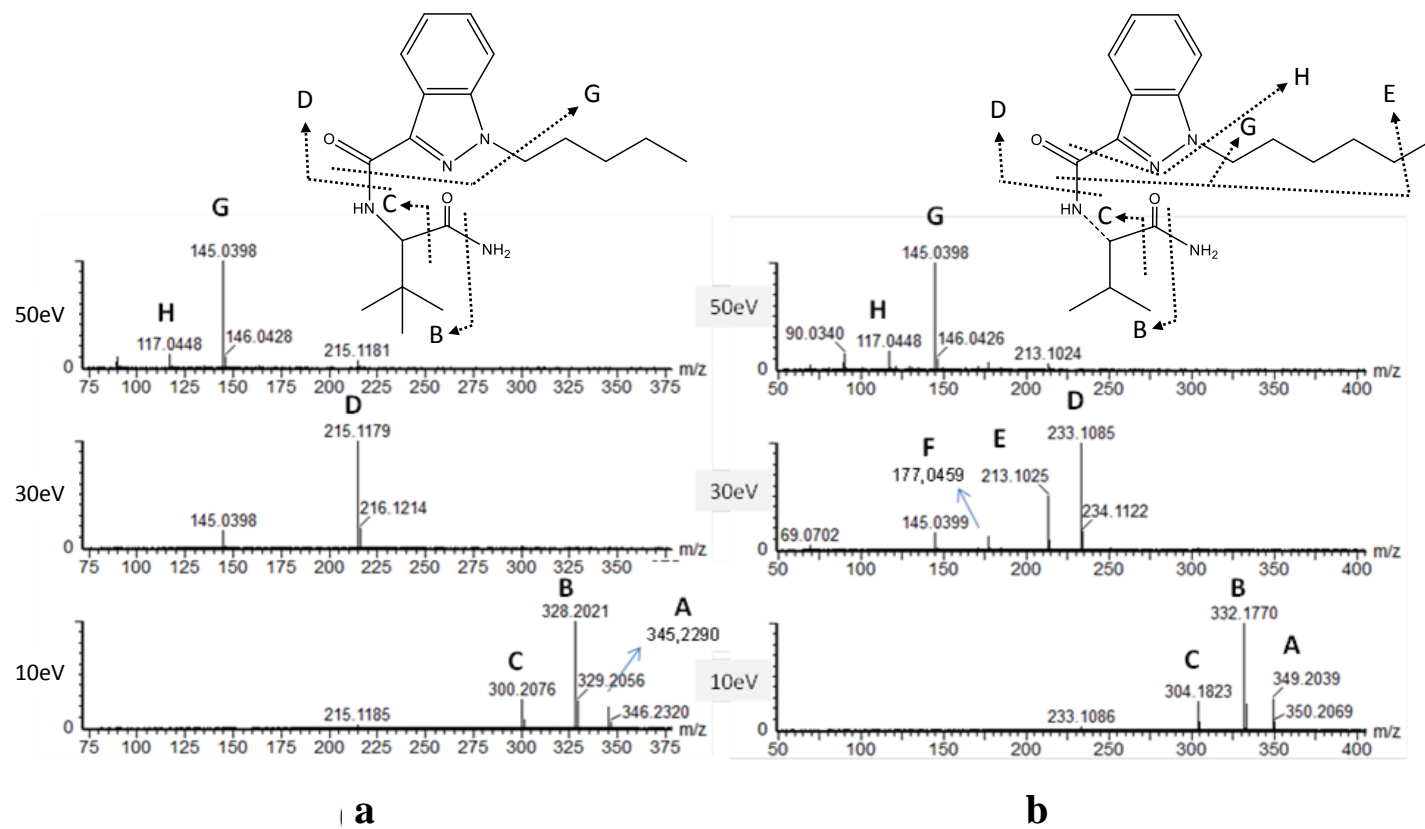


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497 **Fig. 3**

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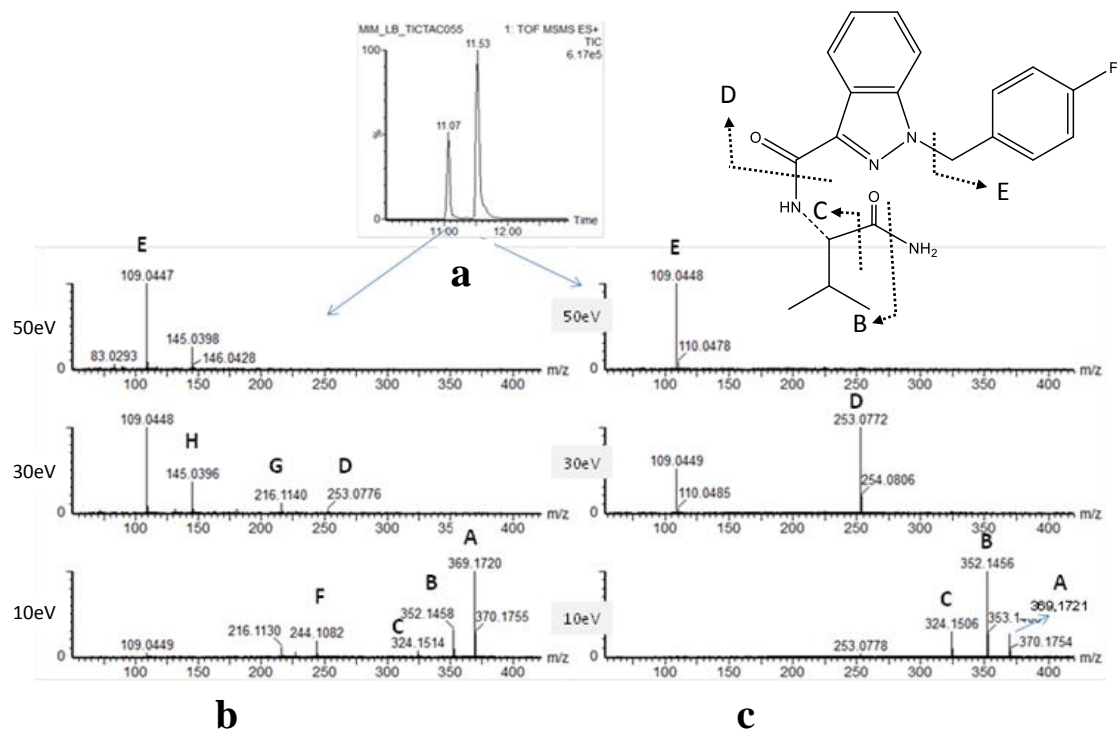


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501 **Fig. 4**

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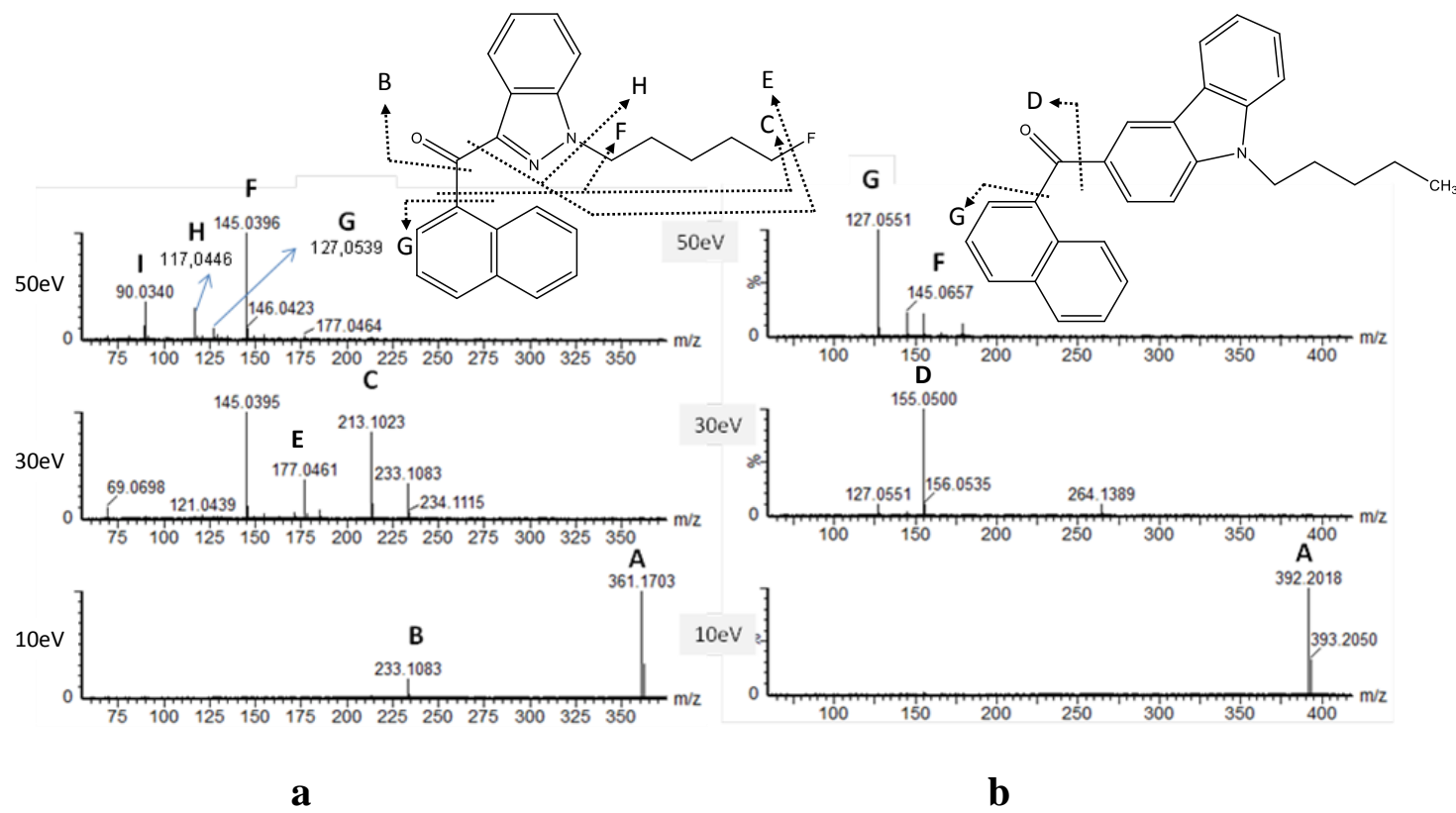
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505 **Fig. 5**

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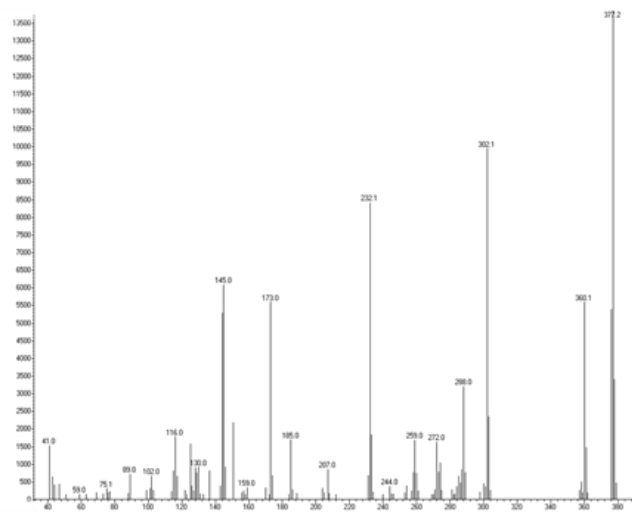


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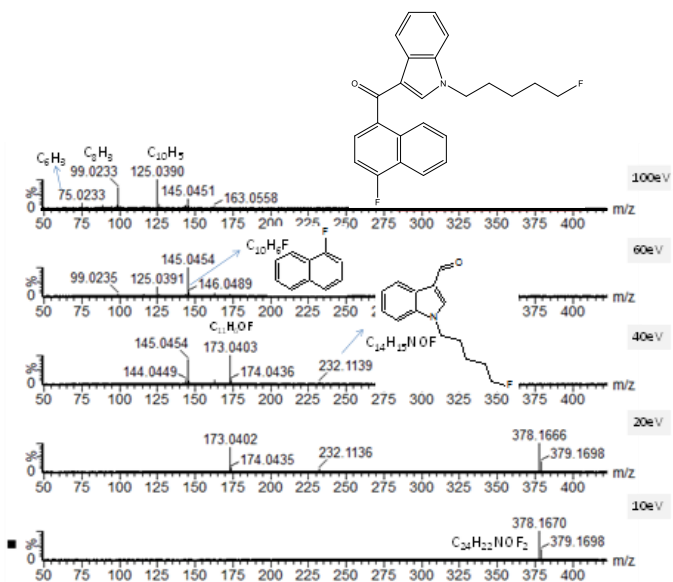
510 **Fig. 6**

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512



a

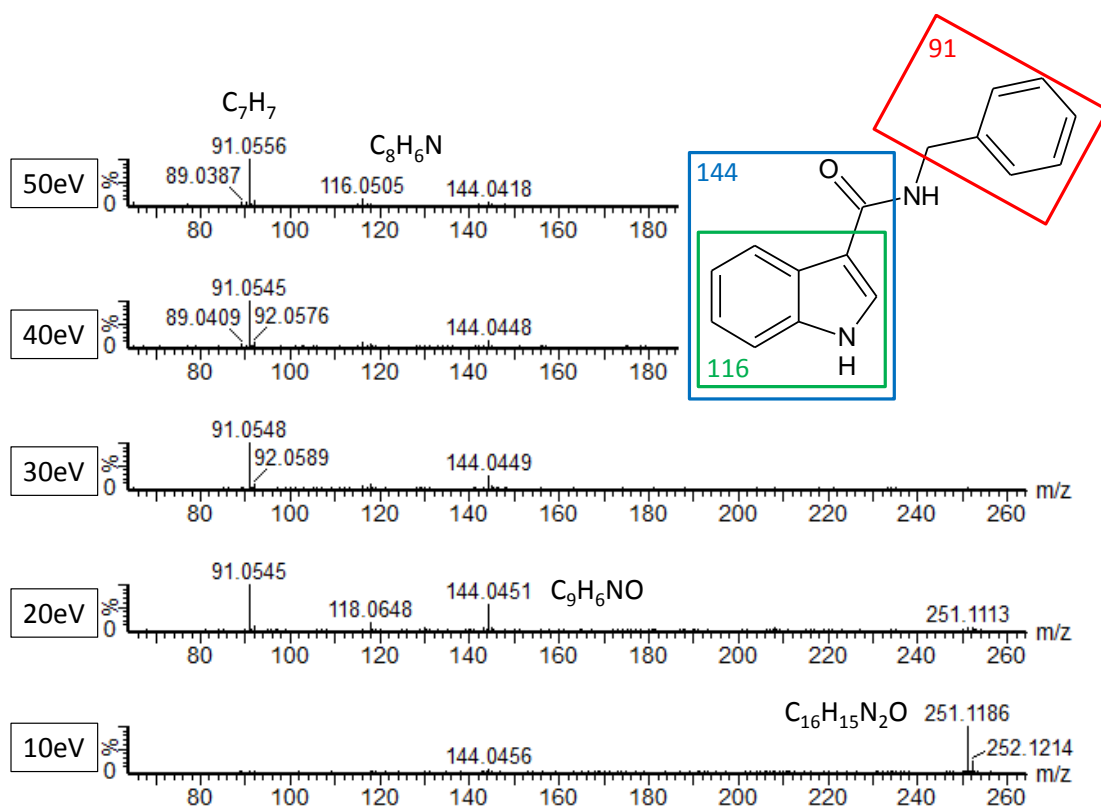


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514 **Fig. 7**

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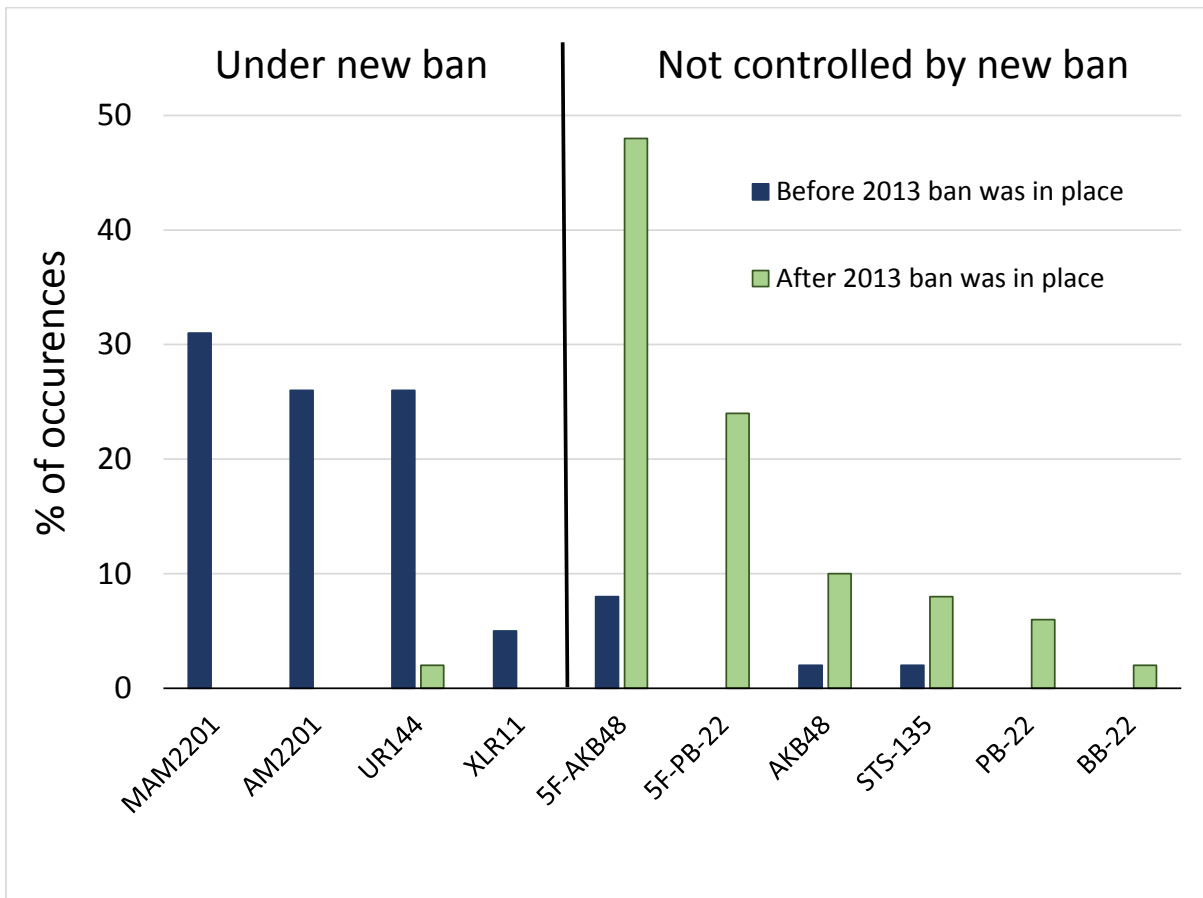


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519 **Fig. 8**

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521

522 **Fig. 9**

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525

526 **Fig. 10**