Isolation, Detection and Identification of Synthetic Cannabinoids in Alternative Formulations or Dosage Forms

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ABSTRACT: Synthetic cannabinoids (SCs) first emerged on the market in head shops in the form of herbal mixtures, and quickly triggered an effort to reclassify them as controlled substances. More recently, with the increased popularity of vaping, new formulations of SCs have emerged. SCs are being dissolved or suspended in e-cigarette liquids and sold as "e-Liquids". Furthermore, suppliers of SCs on impregnated paper sheets have been found on the black market. This could facilitate smuggling of SCs into prisons, exacerbating the problems SCs already create in prisons. The advent of such New Psychoactive Substance formulations/ "new drug-delivery" systems pose a new issue for the detection and control of SCs. This work investigated various SC formulations, including e-cigarette liquids, bulk powder street samples and a set of blank A4 blotting papers believed to be impregnated with SCs to determine qualitatively their drug content. HPLC-DAD, HRMS, NMR and GC-MS provided conclusive structural identification and characterization of a number of different SCs in such alternative forms. Mass- and UV-triggered semipreparative LC-DAD-MS was successfully employed to purify SCs at a mg scale, facilitating their structural identification via NMR, and also paving the way for rapidly obtaining pure SC standards from commercial formulations. Finally, an authentic letter seized from a prison was also analysed and the two synthetic cannabinoids AMB-FUBINACA and MMB-CHMICA were detected. Our data further highlight recent reports about emerging trends which include applying SCs in alternative formulations (i.e. impregnated paper letters and e-liquids) and provide a database to help others identify these potentially toxic substances.

KEYWORDS: synthetic cannabinoids, e-liquid, blotting paper, AMB-FUBINACA, MMB-CHMICA

1.0 Introduction

New Psychoactive Substances (NPS) are a group of recreational drugs that may possess parts of chemical structures and/or pharmacological activity similar to those of traditional controlled drugs. Usually consisting of modified chemical structures of illicit substances, NPS may retain or enhance the ability to produce psychoactive effects and may be easily distributed as "legal highs" [1-3]. Synthetic cannabinoids (SCs), one of the most prevalent drug class of NPS, hit the market in the early 2000s. SCs are compounds that typically possess high affinity to cannabinoid receptors (CB_1 and CB_2) in the human body, thus potent cannabis-like effects are likely to occur when abused. Health-related problems such as cardiovascular and psychological disorders have been associated with their use. Furthermore, their toxicity might be increased by their metabolites and pyrolysis products [4]. In addition to CB_1 and CB_2 receptors, the first generation SCs, including JWH-018, were also reported to have a weak binding affinity (~100 nanomolar) toward non-CB receptors (i.e. serotonin and GABA receptors)[5], which could enhance the complexity of their effects. SCs have been sold in several dosage forms including powders, pellets, and the most prevalent form – herbal blends (e.g. "Spice", "K2") in which the SC is dissolved in a suitable solvent such as acetone, and sprayed onto an inert botanical matrix, followed by solvent evaporation [2, 6].

Recently, the introduction of vaping/electronic cigarette devices (e-cigarettes), has led to a new formulation/delivery of SCs, namely 'e-liquid' preparations. E-cigarette devices constitute a replacement for traditional cigarettes, however, their role in smoking cessation and their safety is still controversial [7, 8]. These devices generate an aerosol from a solution typically containing glycerol and propylene glycol mixtures, nicotine and aromatizing agents/flavoring compounds. The solutions, "e-liquid vapes" or simply "e-liquids" in the cartridge of e-cigarettes are heated to produce an aerosol that is inhaled by the user. ('vaping'). Across the recreational drug market, cannabis or Δ^9-tetrahydrocannabinol (THC) has been found as an active ingredient of some e-liquids e.g. in the form of concentrated oily THC extracts [9]. SCs have been reported to be used in e-liquids by users replacing THC as the psychoactive ingredient [10]. Very recently, the SC MDMB-FUBINACA [11], 5F-CUMYL-PINACA [12] and 5F-ADB [13] have been found in commercial e-liquid preparations. MDMB-FUBINACA and 5F-CUMYL-PINACA in commercial e-liquids have been identified using techniques such as Direct Analysis in Real Time MS, Gas Chromatography-Mass Spectrometry (GC-MS) and Nuclear Magnetic Resonance spectroscopy (NMR). An aerosol produced from MDMB-FUBINACA was also studied using headspace and solid phase micro-extraction coupled with GC [11], 5F-ADB has been unexpectedly detected in e-liquids, that are commercially sold as cannabidiol e-liquid [13].

In recent years, new hallucinogens (e.g. the NBOMe derivative drug class) have been found deposited and distributed on paper sheets [14]; this trend seems to have gained popularity with an increasing number of SCs been detected in this form [15-17]. The 2018 European Drug Report [18] indicates that the use of herbal smoking blends containing SCs in prisons is of particular concern, especially in the UK [17]. Additionally, letters impregnated with NPS are an emerging approach for smuggling SCs into prisons as these can be easily sprayed onto paper[19]. 5F-APINACA, MDMB-CHMICA and AB-FUBINACA are third generation SCs which were found impregnated on five sheets of letters seized from a prison [15]. The drugs were identified by liquid chromatography-high resolution mass spectrometry (LC-HRMS) after the letters were screened for NPS by trained-search dogs [15].

This new and dangerous trend in emerging formulations and dosage forms facilitate both discreet administration (e.g. vaping) and smuggling of SCs in prisons (e.g. impregnated letters) is an ongoing challenge in the SC area. This is because new SCs and the complexity of different formulations may be encountered, complicating their detection and identification. An additional challenge is given by the lack of reference materials to aid the analytical workflow. In this work a batch of suspected SC formulations/dosage forms, including blank A4 papers and several commercial e-cigarette liquids, were analytically characterized to identify their unknown active ingredients. We have exploited an approach that made use of a complementary analytical approach to isolate, detect and confirm these SCs even in the absence of matching reference material

Firstly, the SCs were isolated in pure form using semi-preparative liquid-chromatography-diode array detector-mass spectrometry (LC-DAD-MS) and fully characterized by a suite of analytical techniques such as GC-MS, HRMS and NMR. The retrieved information could also be used to augment existing NPS databases.

Overall, this work aimed at shedding light on recent and emerging SC dosage forms in order to update and inform public health agencies and drug testing laboratories about the diverse formulations/dosage forms now on the market. We show additional evidence of a wide range of SCs in those dosage forms with comprehensive chemical characterization and a simple but effective SC purification process

2.0 Materials and Methods

Samples were test-purchased online from "legal-highs" websites prior to the 26 May 2016 UK blanket NPS ban. A set of e-liquid samples (6 brands, 9 samples) was also test-purchased from online retailers, prior to the ban. All had been sold as cannabinoid-containing mixtures, although the exact cannabinoid compound quoted varied between mixtures or, in some cases, was not disclosed. Three powder samples were supplied by TICTAC Communications Ltd. from street seizures. Finally, three blank A4 sheets of cannabinoid

sprayed/impregnated blotting paper ("blotting paper 01-03") were obtained from an online retailer, the material was not accompanied by any specification about the type of drugs or their amounts. Samples were kept in a locked cabinet at room temperature prior to analysis.

Methanol, acetonitrile, water, formic acid (FA) and trifluoroacetic acid (TFA), HPLC grade, dimethyl sulfoxide-d6, isotopic purity 99.9 atom %D, methanol-d4, isotopic purity 99.8 atom %D, t-butyl-methyl-ether (TBME), tripelennamine hydrochloride, and quinolone were all from Sigma-Aldrich (Dorset, UK).

Sample preparation

Initial screening by HPLC-DAD: samples were dissolved in HPLC mobile phase (50 % water/50 % acetonitrile containing 0.1 % TFA), to a concentration of 100 µg/mL and liquid samples were diluted at a ratio of 1:20 neat e-liquid to mobile phase. E-liquids were easily miscible in mobile phase simply by being vortexed and ultrasonicated for a short period of time. Powder samples proved more difficult to dissolve in mobile phase and required approximately 20-30 minutes of ultrasonication with brief intervals of vigorous vortexing. Blotting paper samples were prepared using 2 cm^2 of paper incubated at room temperature in 5 mL methanol for 20 minutes whilst ultrasonicating; subsequently, the solution was evaporated and reconstituted with 1 mL HPLC mobile phase.

Figure 1. Picture showing the range of e-liquid samples and an A4 sheet of paper sprayed or impregnated with SCs.

Semi-preparative LC-DAD-MS: Liquid samples (1 mL) were diluted to 5 mL with a preparative-LC-DAD-MS-mobile phase (50 % acetonitrile in water containing 0.05 %TFA, 0.1 %FA). 4 cm^2 of paper was incubated in 5 mL methanol for 20 minutes whilst ultrasonicating. The solution was then evaporated and reconstituted with 5 mL of the preparative-LC-DAD-MS-mobile phase.

HRMS analysis: purified samples from semi-preparative LC-DAD-MS were prepared (1 mg/mL, w/v) in 50:50 (v/v) water: methanol containing 0.1 % FA, and diluted to 1 µg/mL. Polypropylene (PP) syringe filters, pore size 0.2 µm, were used to filter samples prior to MS analysis.

NMR spectroscopy analysis: dried, purified samples from semi-preparative LC-DAD-MS were dissolved in deuterated solvents (methanol-*d*4 or dimethyl sulfoxide-*d*6). Solutions were filtered through PP syringe filters.

GC-MS analysis: purified samples obtained from the semi-preparative LC-DAD-MS were reconstituted with 1 mL of methanol which was then vortexed for at least 30 minutes before being centrifuged at 6,030 g for 1 minute. An aliquot of 10 µL was diluted with 1 mL of TBME, containing 100 µg/mL each of quinoline and tripelennamine as the internal standards.

Analytical Instruments

HPLC-DAD: An HP 1050 series equipped with a quaternary pump, an auto-sampler, a diode array detector (DAD) and a Kontron DEG 104 degasser was used. Agilent ChemStation software version A.10.02, was employed to control the equipment. A C8 Kinetex® column 100 × 2.1 mm i.d., particle size 5 µm was purchased from Phenomenex Inc. HPLC mobile phases A and B consisted of 0.1 % v/v TFA in water and acetonitrile, respectively. The gradient was started at 50 % B and ramping from 50 % B to 90 % B from 5 to 20 minutes. The flow rate was set at 0.2 mL/min and the injection volume was 5 µL. The detection UV wavelength was set at 214, 220, 230, 254, and 281 nm. HPLC-DAD was used only for the analytical screening to assess the presence of SCs in each sample by comparing their retention times without using reference materials.

Semi-preparative LC-DAD-MS: Samples were purified using a Waters 2545 Binary Gradient Module, alongside a Waters System Fluid Organiser, a Waters 515 HPLC Pump, a Waters Micromass ZQ mass spectrometer, a Waters 2767 Sample Manager collection system and MassLynx 4.1 software with mass-triggered fraction collection. A chromatographic separation was achieved with an XTerraPrep RP-18 particle size 5 µm, 10 x 150 mm column and LC mobile phases A and B consisting of 0.1 % v/v FA, 0.05 % v/v TFA in water and acetonitrile, respectively. The gradient started at 10 % B and linearly ramped to 90 % B within 50 minutes. The flow rate was set at 10 mL/min and the injection volume was 5 mL.

HRMS: A Thermo Scientific™ Q Exactive™ high resolution high mass accuracy (HRMS) mass spectrometer was employed in direct infusion mode to the mass spectrometer operating either in full scan or MS/HRMS mode for product ion scans. Mass accuracy window of SCs and their fragments falls within 5 ppm. HRMS and MS/HRMS were employed for structural characterization.

UHPLC-HRMS: An Ultimate 3000™ HPLC system coupled to a Q-Exactive™ high resolution mass spectrometer (LC-HRMS) (Thermo Scientific, San Jose, USA) was used only the analysis of the prison letter. It operated under electrospray positive mode, full scan MS range = m/z 100–1000 with resolution (FWHM) = 70,000, AGC target = 1e6. MS^2 for targeted analysis was performed in parallel reaction monitoring (PRM) mode, MS range m/z 100–750 with resolution (FWHM) = 35,000, AGC target = 1e6. A 5

ppm extraction window was set for the extracted ion chromatograms of the protonated ([M+H]$^+$) precursor and product ions. The LC chromatograms and the MS/MS spectra obtained were compared with a previously built in-house library by using the following identification criteria: retention time difference of <2 %, MS/MS relative abundance within ±20 %.

NMR spectroscopy: A Bruker Avance DRX 400 MHz NMR spectrometer was used employing solvent residual peaks as internal standards. Chemical shifts (δ) are quoted in ppm, to the nearest 0.01 ppm (for ^{1}H NMR spectra) and are referenced to the residual solvent peak (CD_3OD). Data are reported as follows: chemical shift, multiplicity (br= broad; s= singlet; d= doublet; t= triplet; m= multiplet, or as a combination of these, e.g. dd, dt, etc.), integration and assignment. Diastereotopic protons are assigned as CH-*H*. ^{1}H NMR was performed to further characterize and confirm SC structures.

GC-MS: The samples (1 µL of the extract) were qualitatively analysed using an Agilent 7890A GC with 5975C VL MSD (Santa Clara, California, USA) equipped with a split-splitless injector (5:1 split ratio) and an HP5-MS column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) and running on Agilent ChemStation software. The column was held at 80 °C for 4 minutes and then ramped up at 40 °C/min to 290 °C and held for a total run time of 39.25 minutes. A mass range of m/z 40 to 400 was scanned. The mass spectrum of the identified analyte was compared with TICTAC in-house library and Cayman GC-MS library in order to confirm the structural characterization. This included analysis of product ion patterns and product ion relative ratios.

3.0 Results and Discussion

Analytical Screening

All samples were analysed with HPLC-DAD using a reversed-phase C8 chromatographic column to assess the presence of chromophore-containing species due to the possible presence of SCs. The method was performed in order to screen for the primary active ingredients of the samples but not for structural characterization.

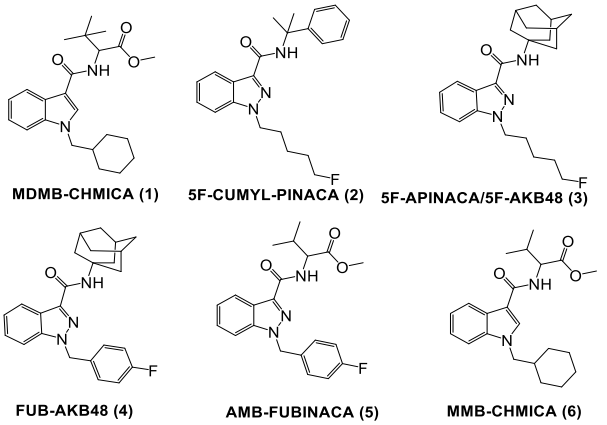

Figure 2. Chemical structures of the detected SCs.

Certain groups of e-liquid products showed similarities in the elution time of the main peaks (**Figure 3**). Liquid samples named “C-liquids” (several flavors) and “Kronic Juice Tropical” presented a main peak at the same retention time which suggested the presence of a common main component. Similarly, the bulk powder of 5F-AKB48, as well as e-liquids of “Buddha Blue” (several flavors), “Sweet Obliteration” and “Happy Joker” showed a main peak at 13 minutes, whereas the chromatograms of two sheets of blotting paper-01 and -03 and “Amnesia” e-liquid presented an analytical peak at around 11 minutes. Three components in FUB-AKB48 and FUB-AMB powder forms and blotting paper-02 eluted at a disparate retention time from other samples, indicating potentially different active ingredients. **Figure 3** shows the HPLC chromatograms of all the samples, separately illustrated according to their formulations. Under the same dilution conditions of e-liquid samples, C-liquids and Kronic Juice Tropical had significantly lower peak intensities compared with Amnesia, Buddha Blue, Sweet Obliteration and Happy Joker. Although extinction coefficients were not taken into account, since SCs normally share similar chromophores, it is reasonable to hypothesize that the various e-liquid formulations were “spiked” with significantly different amounts of SCs. All the chromatograms showed some level of impurities in the samples. Impurity peaks can be observed minutes after the injection in all the chromatograms, which can be assumed not to belong to the SCs given their very lipophilic nature. It is possible that such peaks are due to either flavoring agents, nicotine or other impurities, which were not investigated further in this study. Nevertheless, this demonstrates the need for purification before any of the samples can be used in further analytical (e.g. as reference materials) or biological studies (e.g. *in-vitro* metabolism, or bioassays). Six different cannabinoids in three formulations were putatively classified by HPLC-DAD, and mass-triggered semipreparative LC-DAD-MS was employed for their purification and isolation. The samples were then subsequently subjected to confirmatory analyses via GC-MS, NMR and MS/HRMS.

Structural Identification using High Resolution Mass Spectrometry

Samples were infused into a HRMS using positive ESI mode, in order to determine the accurate masses and elemental composition of the main SC components and confirm their structures by their mass fragmentation using product ion scans in high resolution mode. From these analyses, six SCs in different formulations could be confirmed from the initial screen via HPLC-DAD. **Table 1** shows the classification of six SCs from fifteen samples and accurate masses of all SCs that match with the predicted exact masses with errors being within 3 ppm.

The six SCs included MDMB-CHMICA **(1)**, found in Amnesia e-liquid sample and blotting paper sheets-01 and -03. 5F-CUMYL-APINACA **(2)** was detected only in the e-liquid formulation named “C-liquid” (all flavors) and “Kronic Juice Tropical”. 5F-APINACA **(3),** also known as 5F-AKB48, was detected in seized powder and the e-liquid samples named “Buddha

Blue" (all flavors), "Sweet Obliteration" and "Happy Joker". FUB-AKB48 **(4)**. FUB-AMB powder sample contained AMB-FUBINACA **(5)** and the blotting paper-02 was found to have been impregnated with MMB-CHMICA **(6)**, which is an MDMB-CHMICA analogue. Full scan mass spectra of all SCs was able to detect their $[M+H]^+$ ions together with their respective sodium adducts, $[M+Na]^+$ as monomers and dimers, $[2M+Na]^+$. MS/HRMS product-ion spectra were acquired to investigate the fragmentation of the identified SCs. The preferred fragmentation pathway of most SC molecular ions in this study occurred via the cleavage of their amide bond. However, SCs that contain adamantyl moieties also displayed an adamantyl group as one of the dominant fragments. Product ion spectra of selected SCs detected in this study are shown in **Figure 4**. Structural characterization of six cannabinoids in three formulations samples are shown in **Table 2**.

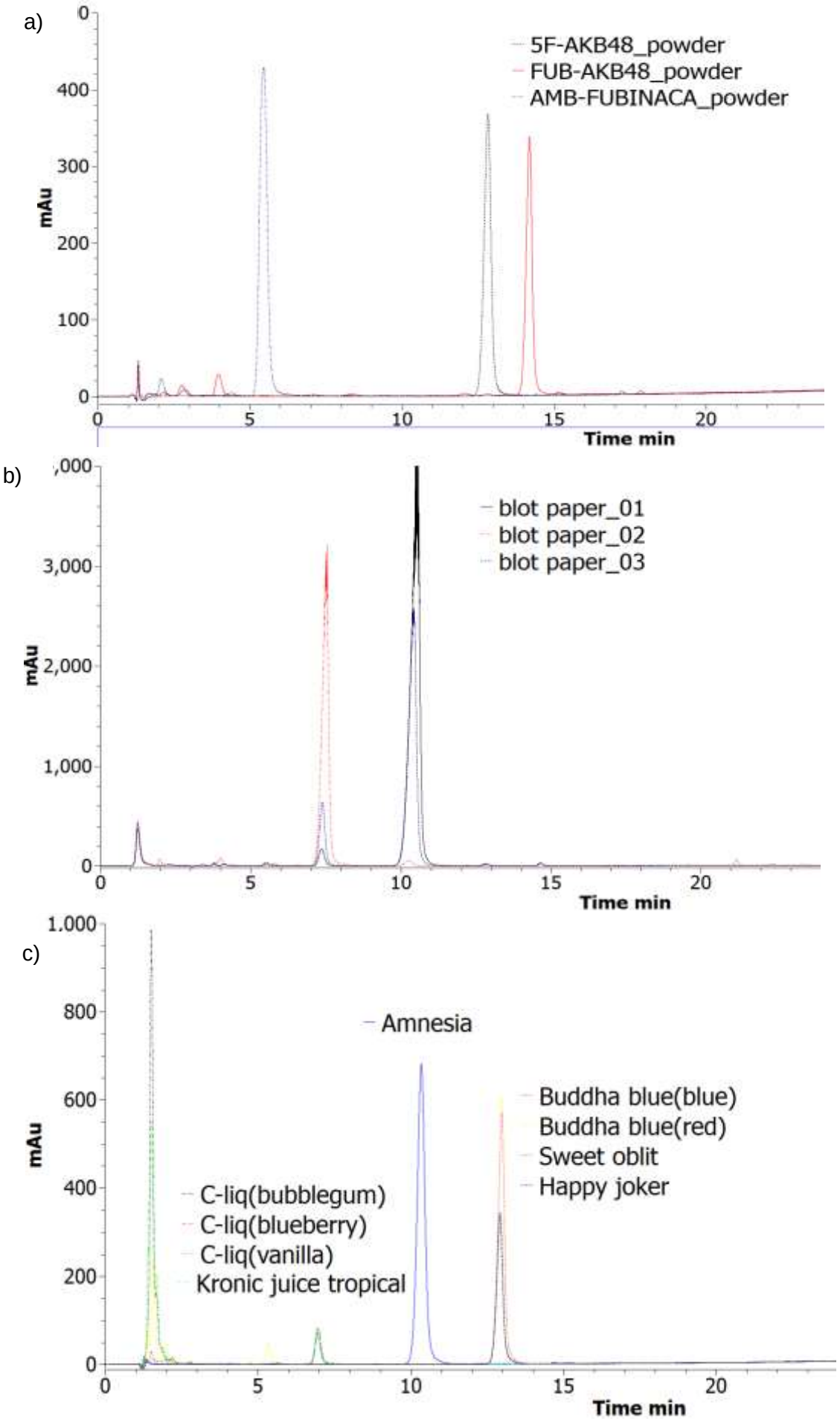

Figure 3: HPLC-DAD chromatograms at 254 nm displaying the analytical screen of samples. (a) powders, (b) blotting papers, (c) e-liquids.

Table 1. Summary of products analysed in this study including the information provided by the vendor.

Commercial name	Cannabinoid Present	Sample form	Additional notes (Ingredients as listed on packaging)
Amnesia	MDMB-CHMICA **(1)**	E-Liquid	- Cannabinoid 0.1 mg/mL - Propylene glycol - Vegetable glycerin
C-Liquid (Bubblegum)	5F-CUMYL-PINACA **(2)**	E-Liquid	N-cumyl-1-(5-fluoropentyl) indazole-3-carboxamide 0.21% w/w
C-Liquid (Blueberry)	5F-CUMYL-PINACA **(2)**	E-Liquid	N-cumyl-1-(5-fluoropentyl) indazole-3-carboxamide 0.21% w/w
C-Liquid (Vanilla)	5F-CUMYL-PINACA **(2)**	E-Liquid	N-cumyl-1-(5-fluoropentyl) indazole-3-carboxamide 0.21% w/w
Kronic Juice Tropical	5F-CUMYL-PINACA **(2)**	E-Liquid	- N-cumyl-1-(5-fluoropentyl) indazole-3-carboxamide - Glycol - Flavouring
Buddha Blue (Blue)	5F-APINACA **(3)**	E-Liquid	N/A
Buddha Blue (Red)	5F-APINACA **(3)**	E-Liquid	N/A
Sweet Obliteration	5F-APINACA **(3)**	E-Liquid	- N-(adamantan-1-yl)-(4-fluorobutyl)-1H-indazole-3-carboxamide - (1R,2S,5R)-2-isopropyl-5-methylcyclohexanol - Propane-1,2,3-triol - Propylene glycol - Aqueous - Flavors
Happy Joker	5F-APINACA **(3)**	E-Liquid	- N-(adamantan-1-yl)-(4-fluorobutyl)-1H-indazole-3-carboxamide - (1R,2S,5R)-2-isopropyl-5-methylcyclohexanol - Propane-1,2,3-triol - Propylene glycol - Aqueous - Flavours
5F-AKB48	5F-APINACA **(3)**	Powder	-
FUB_AKB48	FUB-APINACA **(4)**	Powder	-
FUB_AMB	AMB-FUBINACA **(5)**	Powder	-
Blotting paper-01, 03	MDMB-CHMICA **(1)**	Paper	-
Blotting paper-02	MMB-CHMICA **(6)**	Paper	-

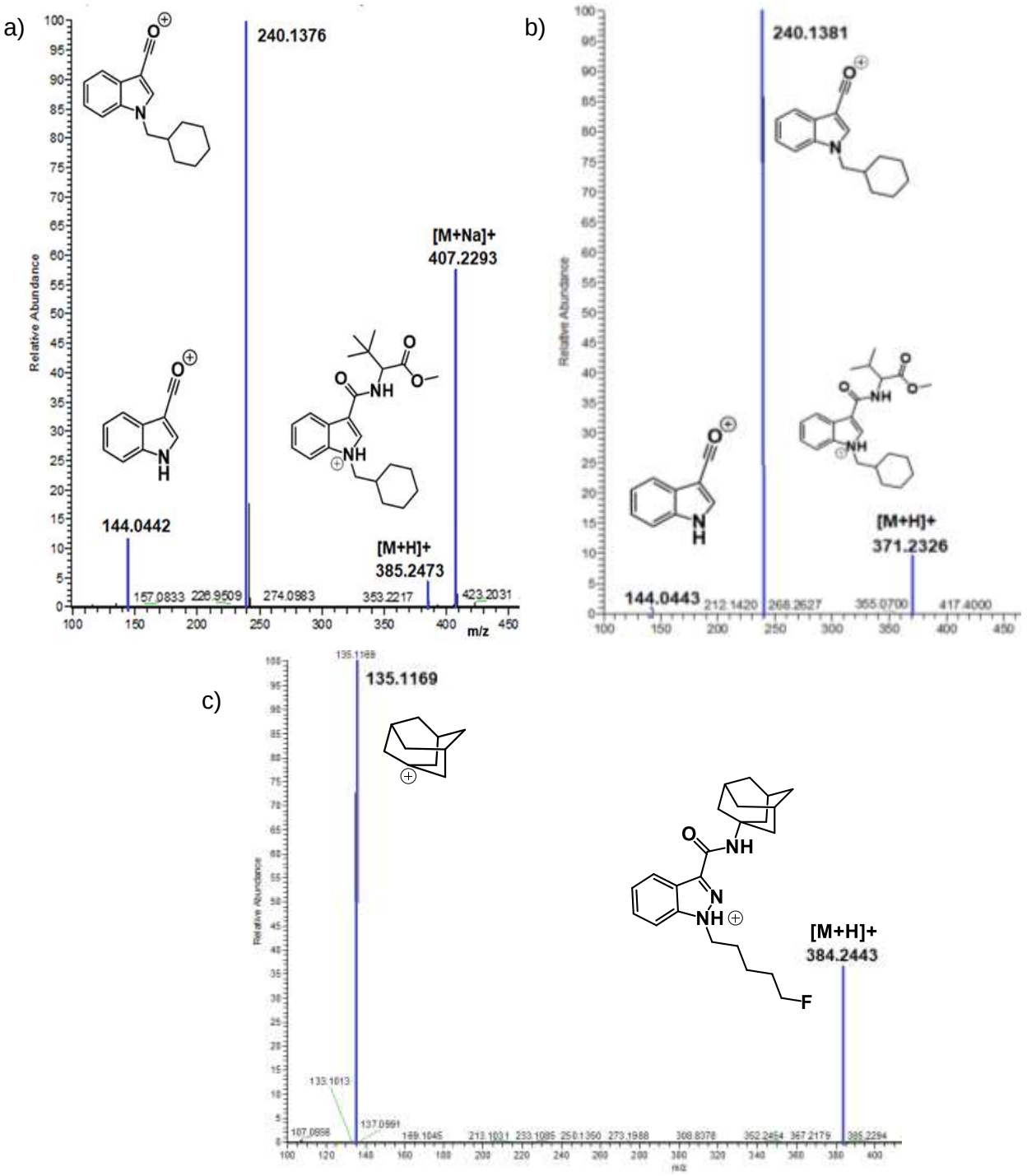

Figure 4. (a) MDMB-CHMICA, (b) MMB-CHMICA and (c) 5F-APINACA product ion spectra of selected samples (Amnesia, blotting paper-02, seized powder).

Structural Identification using NMR

Synthetic drugs produced by clandestine laboratories may contain impurities and by-products [20]. To enable the isolation of pure SCs in milligram scale, samples were injected into a preparative LC/MS purification system with mass-triggered fraction collection prior to NMR analysis. Following the purification, further analysis of key samples was conducted via NMR spectroscopy. **Figure 5** compares the ^{1}H NMR spectra for crude and purified samples from a commercial E-liquid preparation. The spectra demonstrate that the SCs suspected from previous analysis could be confirmed using NMR; and that purification of the samples is essential to obtain a clear, interpretable spectrum, as well as to enable using the isolated compounds as *in-house* reference materials for (bio)-analytical assays.

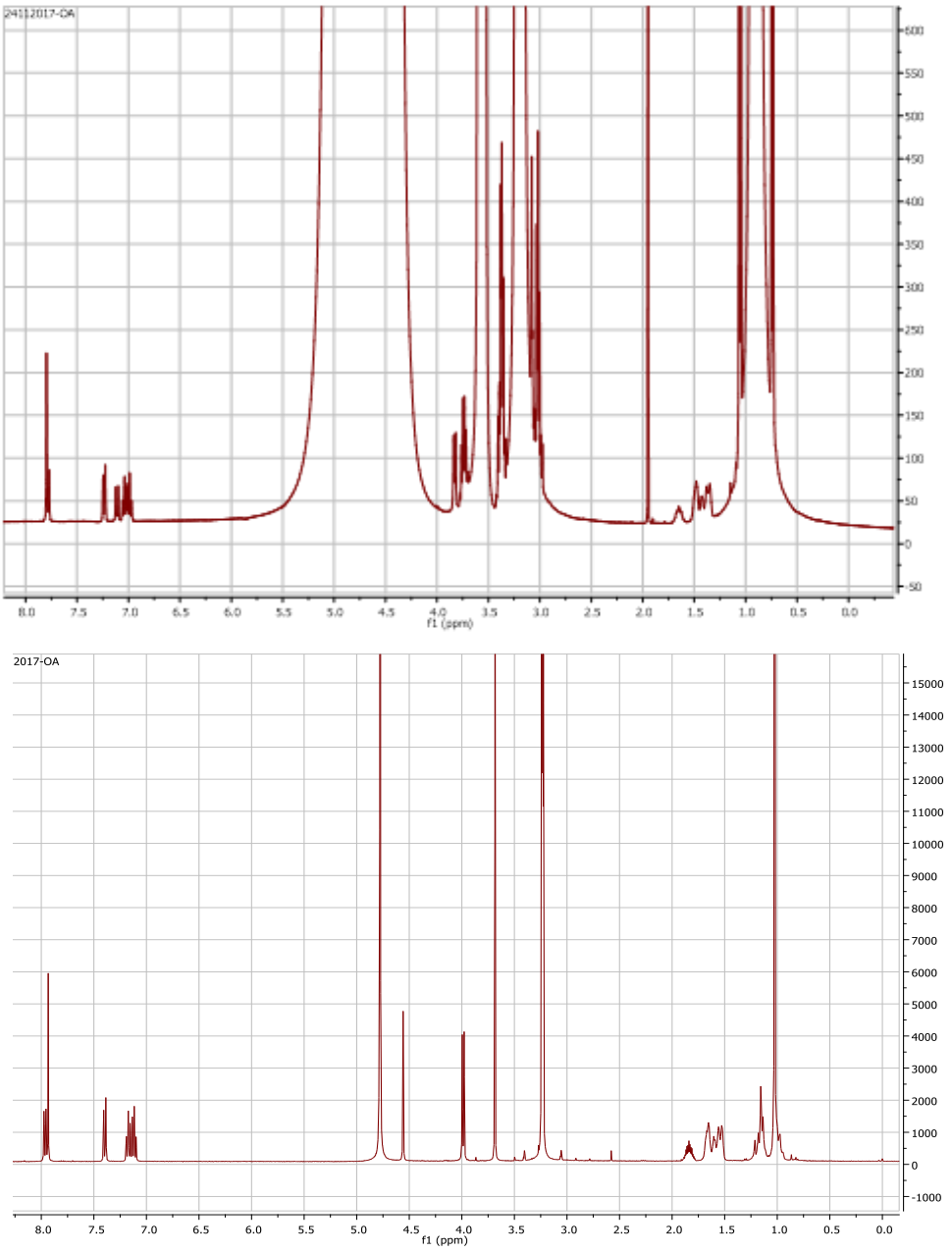

Figure 5: ^{1}H NMR spectrum of Amnesia crude sample (top, showing strong interfering water peak) and Amnesia purified sample (bottom).

NMR characterization of MDMB-CHMICA, MMB-CHMICA and 5F-APINACA were consistent with previously reported spectra in the literature [21-24].

MDMB-CHMICA **(1).**^{1}H NMR (400 MHz, MEOD) δ7.97 (d, 1H, Ar), 7.93 (s, 1H, C_6H_4CCHN), 7.40 (d, 1H, Ar), 7.17 (t, 1H, Ar), 7.12 (t, 1H, Ar), 4.56 (s, 1H, $(CH_3)_3CCHCO$), 3.99 (d, 2H, NCH_2CH), 3.69 (s, 3H, OCH_3), 1.72-1.46, 1.27-1.09 (m, 10H, $5CH_2$), 1.03 (s, 9H, $3CH_3$).

MMB-CHMICA **(6).**^{1}H NMR (400 MHz, MEOD) δ8.10 (d, 1H, Ar), 8.00 (s, 1H, C_6H_4CCHN), 7.48 (d, 1H, Ar), 7.26 (t, 1H, Ar), 7.20 (t, 1H, Ar), 4.56 (d, 1H, $(CH_3)_2CHCHCO$), 4.08 (d, 2H, NCH_2CH), 3.78 (s, 3H, OCH_3), 1.80-1.61, 1.33-1.20 (m, 10H, $5CH_2$), 1.07 (s, 6H, $2CH_3$).

5F-APINACA **(3).**^{1}H NMR (400 MHz, MEOD) δ8.21 (d, 1H, Ar), 7.64 (d, 1H, Ar), 7.46 (t, 1H, Ar), 7.28 (t, 1H, Ar), 4.54-4.44 (m, 3H, $NCH_2CH_2+CH_2CH_2F$), 4.35 (t, 1H, CH_2CH_2F), 2.23 (br, 6H, $C(CH_2)_3(CH)_3(CH_2)_3$), 2.16 (br, 3H, $C(CH_2)_3(CH)_3(CH_2)_3$), 2.07-1.93 (m, 2H, CH_2CH_2F), 1.84-1.66 (m, 8H, $C(CH_2)_3(CH)_3(CH_2)_3+NCH_2CH_2$), 1.49-1.39 (m, 2H, $CH_2CH_2CH_2F$).

GC-MS analysis

Purified samples were run on a GC-MS system to aid confirming their structural identification. The results of the tested samples were compared against in-house libraries that had been previously built up by

TICTAC Communications Ltd. as well as the Cayman GC-MS library [25-27]. Results from the GC-MS analyses showed that the samples of Amnesia (MDMB-CHMICA), blotting paper-02 (MMB-CHMICA) and 5F-AKB48 powder (5F-APINACA) match their SC library entries [25-27]. GC-EI-MS chromatograms of selected samples are presented in **Figure 6**.

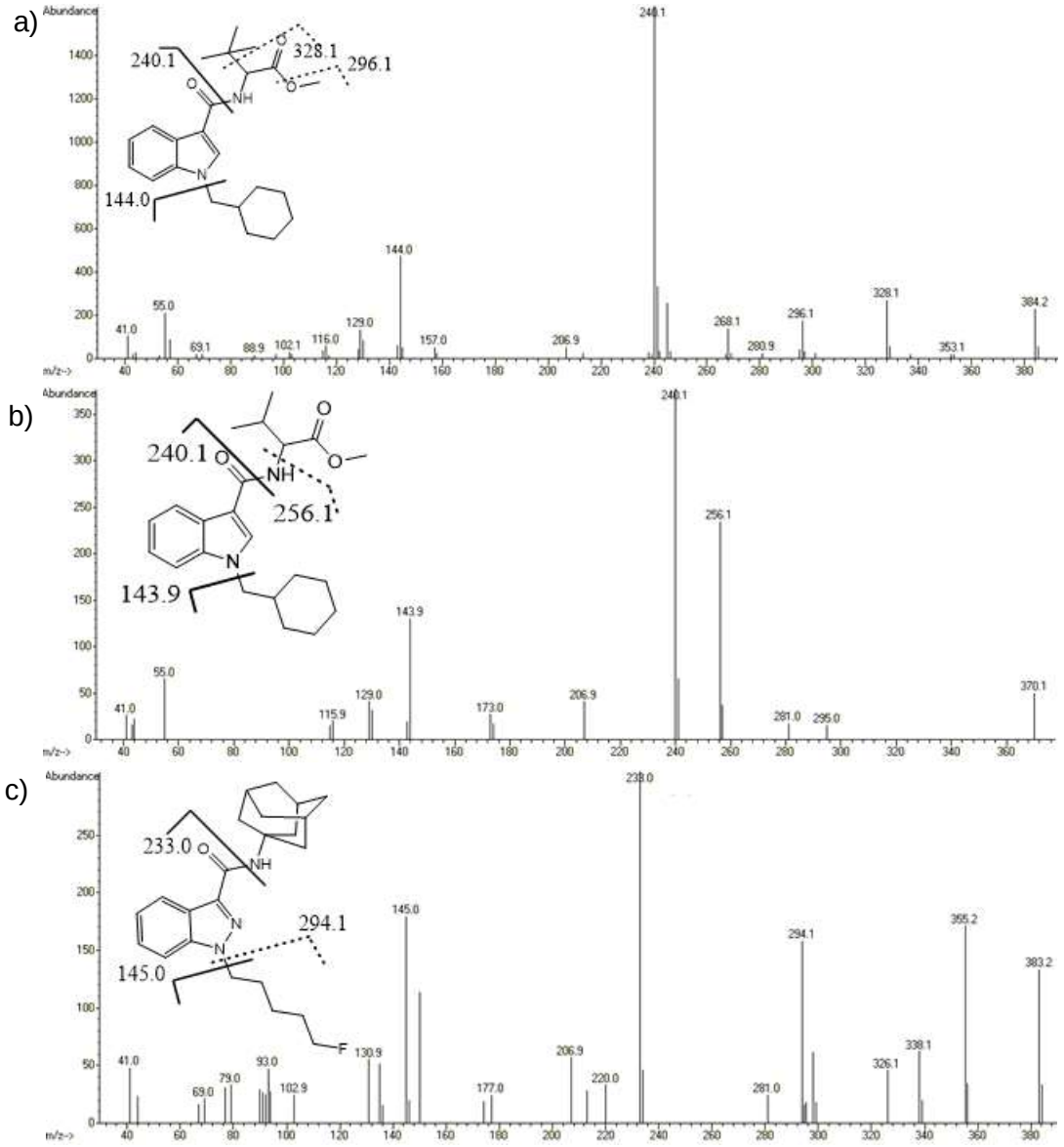

Figure 6. GC-EI-MS of: MDMB-CHMICA in Amnesia e-liquid (a); MMB-CHMICA in blotting paper-02 (b); 5F-APINACA in seized powder (c).

Table 2: HRMS analysis of six cannabinoids in three formulations samples.

Cannabinoids	Sample label	Theoretical mass $[M+H]^+$	Measured mass $[M+H]^+$	Mass error (ppm)	Observed fragment ions (m/z)
(1) MDMB-CHMICA	- Amnesia - Blotting Paper-01, -03	385.2486	385.2485	-0.26	144.0444, 240.1380
(2) 5F-CUMYL-PINACA (SGT-25)	- C-Liquid (all flavors) - Kronic Juice Tropical	368.2133	368.2124	-2.44	119.0853, 145.0395, 233.1080
(3) 5F-APINACA	- Buddha Blue (all flavors) - Sweet Obliteration - Happy Joker - 5F-AKB48	384.2446	384.2444	-0.52	135.1169, 233.1082, 250.1349,
(4) FUB-APINACA	FUB-AKB48	404.2133	404.2136	0.74	135.1169, 227.0981, 253.0774
(5) AMB-FUBINACA	FUB-AMB	384.1718	384.1716	-2.34	109.0449, 253.0771, 324.1505
(6) MMB-CHMICA	Blotting Paper-02	371.2329	371.2330	0.27	144.0451, 240.1381

Analysis of NPS impregnated prison letter

We performed the analytical characterization of a letter seized from a prison to identify a suspected SC drug laced on via spraying and/or impregnation from a SC solution. (**Figure 7**). Two SCs, AMB-FUBINACA (**5**) and MMB-CHMICA (**6**) were detected on prison letter samples and characterized via UHPLC-HRMS. AMB-FUBINACA and MMB-CHMICA were detected by full scan LC-HRMS and their structures confirmed via targeted MS/HRMS product ion spectra.

Fragment ions at m/z 324.1488, 253.0759 and 109.0442 confirmed the structure of AMB-FUBINACA, whereas those at m/z 240.1376 and 144.0441 were detected from the MS/MS spectrum of MMB-CHMICA (**Figure 8**). These fragment ions were in good accordance with the observed MS/HRMS spectra of AMB-FUBINACA detected in the powder sample and MMB-CHMICA detected in blotting paper-02 (as shown in **Table 2**). The LC-HRMS chromatogram and the MS/MS spectrum of AMB-FUBINACA and MMB-CHMICA matched those reported in a HRMS in-house library.

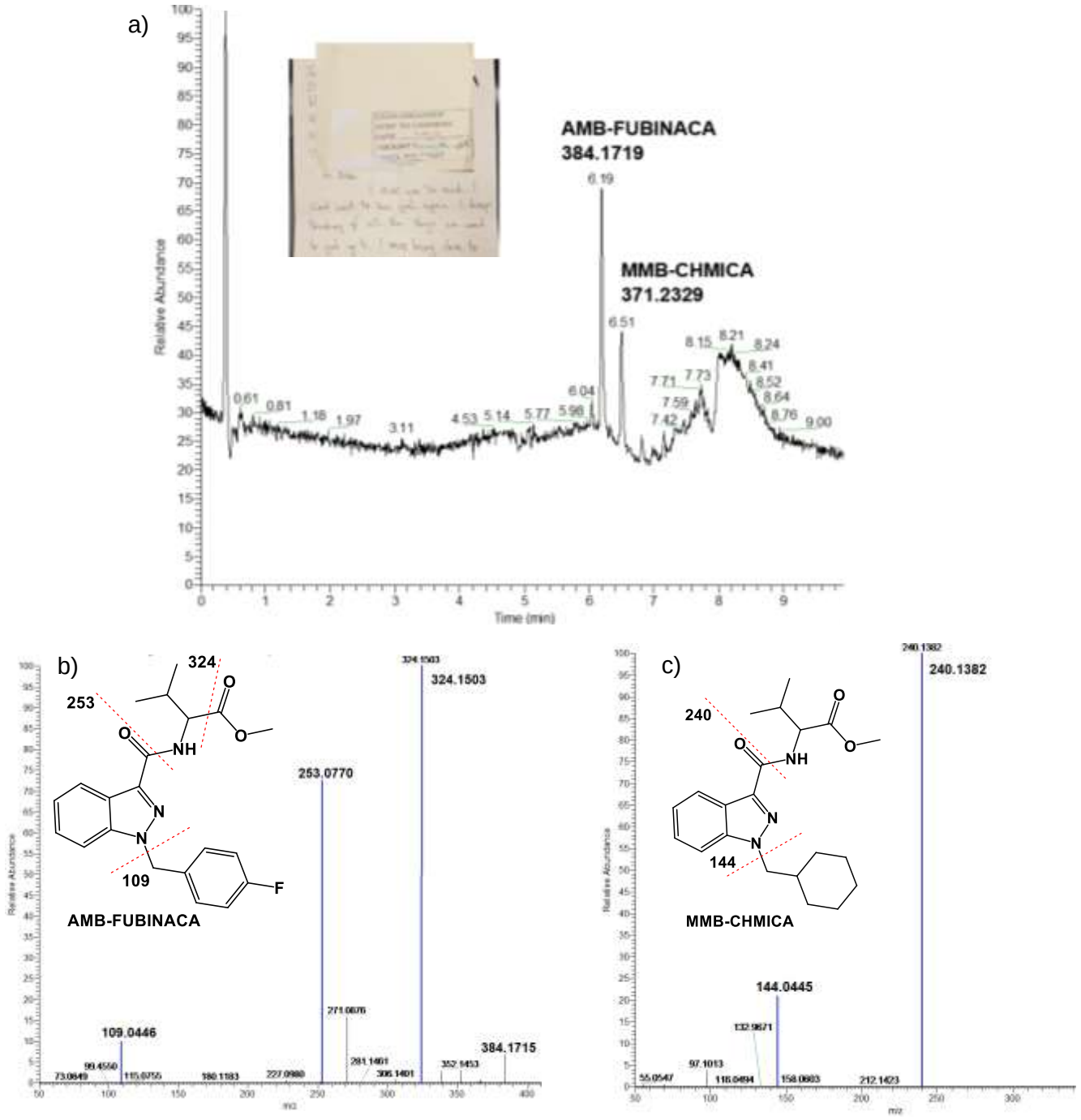

Figure 7. The detection and characterization of two SCs in a seized prison letter: full scan HRMS (a); MS/HRMS of AMB-FUBINACA (b); MS/HRMS of MMB-CHMICA (c)

4.0 Conclusions

In this study, three different SCs were detected and characterized from six brands of e-liquids. Similarly, three sheets of purchased “blotting paper” were found laced with two types of SC. And two cannabinoids were detected from a seized prison letter. In total, six unique SCs were detected: MDMB-CHMICA **(1)** was present in one e-liquid – Amnesia – and the blotting paper sheets-01 and -03. MDMB-CHMICA in Amnesia e-liquid displayed a disproportionately high concentration compared to other e-liquid samples. The current literature primarily declares misuse of SCs in herbal mixtures, therefore this report is one of the few to have fully described MDMB-CHMICA in an e-liquid, and the first ever report of SC (MDMB-CHMICA and MMB-CHMICA)–impregnated on a blank paper sample purchased on the Internet. 5F-CUMYL-PINACA **(2)** was detected in two liquid samples – Kronic Juice Tropical and C-liquid. The compound was recently reported and studied in literature [12, 28]. 5F-APINACA **(3)** was detected in the majority of the liquid samples (i.e. Buddha Blue, Happy Joker, Sweet Obliteration) and also in the powder sample. making it the most prevalent SC detected overall and found in diverse dosage forms. Current literature supports this as it has been detected frequently in both powder and herbal samples, however, this report is the first occurrence of it being detected in an e-liquid.

SCs appear in the drug market continuously, not only as new analogues, but also as new product formulation/dosage forms. The primary formulations, herbal mixtures, are now accompanied by e-liquids, which provide a more discreet administration, posing challenges for police, public health authorities and forensic labs. Furthermore, SCs are also sometimes “delivered” as impregnated letters, facilitating smuggling of these drugs in prisons, a process which is currently very popular [17]. Several SCs have been found in different formulations and, without reference material, a complementary methodological approach for their characterization was developed and performed. Also, the use of semi-preparative LC-DAD-MS allowed fast isolation of the active and psychoactive components from its original matrix. This may provide a standardized route to the isolation of SCs from e-cigarette liquids, as opposed to more conventional sample extraction methods such as liquid-liquid extraction.

Such drug scenarios are likely to become more prominent following legislative bans which may slow down the design and marketing of new SC analogues, and could shift the attention of black markets towards innovative and “smart” dosage forms. This reinforces the need for cataloguing specimens in order to be able to identify previously discovered SCs and their legal classification in a simpler way, thereby helping enforcement agencies. This will also allow more focused research on compounds or novel formulations not so catalogued, e.g. in the field of rapid detection and/or new development of the detection. Indeed, such novel formulations are less likely to be seized or controlled by police/prison officials as they are more difficult to detect in visual inspections than a conventional white powder or herbal preparations. This poses a new challenge for human health and constitutes an emerging social problem.

Anecdotal and/or case reports suggest potential serious toxicity from the misuse of SCs, but an understanding of the mechanisms of their toxicology is lacking and needed. Herein, we have illustrated the variety of formulations that are available, and although current legislative bans have reduced the number of NPS notified for the first time to the European Monitoring Centre for Drugs and Drug Addiction[19], the NPS trend may be dangerously shifted from new chemistries to new drug delivery systems. We have also provided forensic community with data that should help with the definitive identification of these potentially toxic compounds.

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