Title:

Source: Eur. J. Med. Chem. 2017, 127, 757–770

Identification of a new tert-leucinate class synthetic cannabinoid in powder and "spice-like" herbal incenses: Methyl 2-[[1-(5-fluoropentyl)indole-3-carbonyl]amino]-3,3-dimethyl-butanoate (5F-MDMB-PICA)

Author(s): Risseeuw, M. D. P.; Blanckaert, P.; Coopman, V.; Van Quekelberghe, S.; Van Calenbergh, S.; Cordonnier, J.

Source: Forensic Sci. Int. 2017, 273, 45-52

Identification of a new *tert*-leucinate class synthetic cannabinoid in powder and "spice-like" herbal incenses : methyl 2-[[1-(5-fluoropentyl)indole-3-carbonyl]amino]-3,3-dimethyl-butanoate (5F-MDMB-PICA)

Martijn D.P. Risseeuw[1], Peter Blankaert[2], Vera Coopman[3], Stijn Van Quekelberghe[3], Serge Van Calenbergh[1] and Jan Cordonnier[3]

[1] Laboratory for Medicinal Chemistry, Ghent University, Ottergemsesteenweg 460, 9000 Gent, Belgium

[2] Belgian Early Warning System Drugs, Scientific Institute of Public Health, Juliette Wytsmanstraat 14, 1050 Brussel, Belgium.

[3] Eurofins Forensics Belgium, Lieven Bauwensstraat 6, 8200 Brugge, Belgium.

* Corresponding author. Tel.: +32 50 31 02 52; fax. + 32 50 31 02 54

E-mail address : jancordonnier@eurofins.be (J. Cordonnier)

Abstract

A brown powder and different product packages of "spice-like" herbal incenses were analyzed using a systematic identification approach based on liquid chromatography with diode array detector (HPLC-PDA) and gas chromatography mass spectrometry (GC-MS) with computer based library search against spectral libraries. However, the most predominant compound in the methanolic sample solutions could not be identified. In order to elucidate the chemical structure, a more extensive analysis of the material was initiated using liquid chromatography mass spectrometry (LC-MS), electrospray high resolution mass spectrometry (ESI-HRMS) and ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance spectroscopy (NMR), which allowed the identification and characterization of the major compound as methyl 2-[[1-(5-fluoropentyl)indole-3-carbonyl]amino]-3,3-dimethyl-butanoate (5F-MDMB-PICA).

The goal of this study is to provide analytical information for the identification of this new *tert*-leucinate class synthetic cannabinoid by various analytical methods.

1.Introduction

The last decade has seen the global appearance of novel drugs with (psychoactive) characteristics comparable to the commonly known illicit drugs of abuse; hence the term new psychoactive substances (NPS) was coined. Contrary to other synthetic abused drugs, most of these NPS have never appeared in academic research, and have never been studied. As a result, very little - if any- information is available regarding mode of action, human/animal

pharmacology, or acute and chronic toxicity. Also typical is that these NPS are being actively sold in internet web shops, and that chemical synthesis is mostly performed under dubious circumstances in ill-equipped Chinese labs. In the EU alone, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reported in its European Drug Report 2016 the monitoring of more than 560 novel substances, of which 380 (70%) were detected in the last 5 years (1). These substances can be classified according to chemical class and/or mode of action.

The largest class of compounds currently monitored by the European Early Warning System consists of synthetic cannabinoids. This class consists almost exclusively of cannabinoid agonists, with high affinities for cannabinoid receptors; both CB1 and CB2 agonists have been reported. The use of these substances has been recorded in several "user groups", such as psychonauts, prisoners and high school students. Easy availability, the legal status, and sometimes low cost have been reported as motives for use of synthetic cannabinoids. Also people who want to avoid detection of illicit drugs (such as people undergoing regular urine drug screening in a judicial context) also report the use of synthetic cannabinoids. In Belgium however, the use of synthetic cannabinoids is generally low, although many compounds are detected and identified every year. The majority is seized by customs while in transit from foreign countries (especially China), and have other EU countries as destination. Data are lacking, but it is estimated that actual use of the compounds in Belgium remains limited, contrary to other EU member states. (2)

These cannabinoids (and NPS in general) are frequently advertised as 'legal highs', and marketed as safe and legal alternatives to cannabis. However, an increasing number of papers is being published describing adverse effects, toxicity, and even deaths caused by the

consumption of synthetic cannabinoids. Although these substances are sold as legal alternatives to (illegal) cannabis, ironically these synthetic cannabinoids can cause severe toxicity that has never been observed after cannabis use. For example, in July 2015 a serious outbreak of intoxications and fatalities after the consumption of a synthetic cannabinoid herbal mixture (called "Mocarz", meaning "Strongman") occurred in Poland. More than 200 users were hospitalized with intoxication symptoms, and several deaths have been reported (3–6). Several different synthetic cannabinoids were identified in "Mocarz" herbal mixtures, and more specifically MDMB-CHMICA seems to have caused a lot of victims (6–9).

Similar events on a smaller scale have also been reported throughout Europe (10), triggering the EMCDDA to perform a formal risk assessments for some compounds, such as MDMB-CHMICA, with subsequent initiatives to schedule the substance at the EU level (11-13).

The analytical detection of NPS remains a challenge for forensic laboratories, moreover, for clinical laboratories or emergency services, identification by means of second level analysis requires reference standard materials, methodologies and analytical equipment that are usually not available to every laboratory yet. Hence, this paper aims to provide a thorough overview of the analytical methods that were used to unequivocally identify the seized substance as 5F-MDMB-PICA (Figure 1). The different used methods (GC-MS, HPLC-PDA, ESI-LC-MS, ESI-HRMS and ^{1}H, ^{13}C and ^{19}F NMR spectroscopy) will be discussed in detail. The results presented here are, to our knowledge, the first report of the analysis and identification of the synthetic cannabinoid 5F-MDMB-PICA from a seized powder and herbal incenses. The results may be used for analytical purposes in the clinical and forensic sciences in order to improve the detection of this substance both on the EU as on the international level.

2. Materials and methods

2.1 Samples and case histories

In November 2016, a powder in an unlabeled zip-locked plastic bag and 27 product packages of Bonzai herbal incenses of different flavors (aluminum foils bags with content: 3 g each), were submitted to the laboratory for toxicological analysis. The powder was brown and appeared as a partially grainy, amorphous solid with a strong and unpleasant odor. It was stated that the powder was used as raw material in the production of the herbal incenses.

2.2 Materials

The solvents used for GC-MS and HPLC-PDA were of analytical grade. Methanol and acetonitrile were obtained from Fisher Chemical (Fisher Bioblock, Belgium). Water was purified by a Milli-Q system obtained from Merck Millipore (Darmstadt, Germany). Triethylammonium (TEA) phosphate buffer 1M was purchased from Sigma (Zwijndrecht, Belgium) and was diluted 1/40 immediately before use. Diphenylamine used as external standard was obtained from VWR International (Leuven, Belgium). Solvents for ESI-HRMS were of ULC-grade and were purchased from Biosolve (Valkenswaard, the Netherlands). Acetonitrile for ESI-LCMS was purchased from Merck (Darmstadt, Germany) and was of HPLC grade. Water was obtained from a Millipore Synergy UV apparatus (Billerica MA, USA). Formic acid was purchased from Sigma-Aldrich and was of mass spectrometry grade. Deuterated dimethyl sulfoxide (0.03%, v/v tetramethylsilane) for NMR was obtained as ampoules (750 µL) from Euriso-Top (Saint Aubain, France).

2.3 Instrumentation and sample preparation

2.3.1   Gas chromatography-mass spectrometry (GC-MS)

For the determination of the mass spectrum of the compound, analysis of a freshly prepared methanolic sample solution (containing 200 µg/ml diphenylamine as external standard) was performed using an Agilent 6890 N gas chromatograph in combination with a Agilent 7683 injector and an Agilent 5973 inert mass selective detector (Agilent Technologies, California, USA). A Varian CP-SIL 8 CB low bleed capillary column (30 m x 0.25 mm, 0.25 µm film thickness) was used connected to a fused silica retention gap (2.5 m x 0.25 mm). Carrier gas was helium at a constant flow of 1.1 ml/min. The temperature program used was as follows: start at 70 °C with 2 min holding time, increase to 310 °C at 8 °C/min, 9 min holding time at 310 °C, total runtime 41 min. The temperatures of the injection port and the detector were set at 300 and 230 °C, respectively. The transfer line temperature was set at 280 °C. The injection volume was 1 µL using the splitless injection mode. Mass spectra were recorded in the range m/z 40-550.

2.3.2   Liquid chromatography with photo-diode array detection (HPLC-PDA)

The UV-spectrum of the compound was obtained by HPLC-PDA. A sample solution of the powder was prepared in methanol (1.198 mg/ml). A 50 µl aliquot was evaporated at 40 °C under a gentle stream of nitrogen and reconstituted in 1.0 ml initial mobile phase (containing 20 µg/ml diphenylamine as external standard). A final concentration of 59.9 µg powder/ml was injected.

A 50 µl aliquot of a crude methanol extract of the herbal incense (homogenized, 246.8 mg/ 20 ml, sonicated for 30 min and centrifuged) was evaporated at 40 °C under a gentle stream of nitrogen and reconstituted in 1.0 ml of initial mobile phase (containing 20 µg/ml diphenylamine). A concentration of 617 µg/ml was injected.

The HPLC-PDA analysis was performed using a Varian Prostar solvent delivery module in combination with a Varian Prostar 410 autosampler and Varian Prostar photodiode array detector. Data acquisition and analysis were performed with the Varian Star and Polyview software. A LiChrospher® 100 RP-18 (5 µm) (Merck, Darmstadt, Germany) was used as saturation column. The separation of compounds was performed in gradient mode with the use of a Microsorb C18 column (150 mm x 4.6 mm, 5 µm particle size, Agilent, California, USA) connected to a C18 guard column (4 mm x 3.0 mm, 3.5 µm particle size). The oven temperature was set at 35 °C. The mobile phases consisted of 25 mM TEA-phosphate buffer (A) and acetonitrile (B). The following gradient elution was programmed: 95 % A at time 0 min, changed to 30 % A in 30 min and held for 5 min. The scan range was 220-340 nm and the chromatogram was monitored at 220 nm and 254 nm for 35 minutes. The injection volume was 50 µl.

2.3.3 Electrospray liquid chromatography mass spectrometry (ES-LC-MS)

A solution of the powder was prepared by dissolving the material in acetonitrile to a concentration of 1.0 mg/ml. A Waters® AutoPurification™ system was equipped with a Waters® CORTECS® column (C18, 4.6 x 100 mm, 2.7 µm particle size) and using Waters® MassLynx software. Optical detection was achieved using a Waters® 2998 PDA. MS-detection was done in positive mode using a Waters® Acquity QDA detector. The mobile phases consisted of water/formic acid (1000:1, v/v) (A) and acetonitrile (B). Elution was

carried out at 1.44 ml/min at 20 °C. The following gradient elution was programmed: 95% A at time 0, changed to 5% A over 10 min and held for 2 min. The injection volume was 5 μL.

2.3.4   Electrospray high resolution mass spectrometry and molecular formula analysis (ES-HRMS)

A solution of the powder was prepared by dissolving the material in acetonitrile to a concentration of 1.0 mg/ml. This solution was diluted 1000x with acetonitrile to a final concentration of 1.0 μg/ml. High resolution mass spectra were recorded on a Waters® LCT Premier XE (TOF) set for electrospray ionization and using Waters® Masslynx software. The mass was referenced using Leucine Enkephalin (Sigma-Aldrich) as an external standard. Spectra were generated in positive ionization mode by direct infusion of the above mentioned solution (10 μL) using an additional flow (150 ul/min) of acetonitrile/water/formic acid (1000/1000/1, v/v/v). After obtaining the high resolution mass value, a statistic ranking of the most probable molecular formulas was generated from a Single-Mass-Analysis using the Elemental-Composition function in MassLynx.

2.3.5 ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance spectroscopy (NMR)

To achieve secure proof of the molecular structure the ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) spectrum of the compound was assigned. A solution of the powder in DMSO-d6 (20 mg in 750μL) was prepared in a Wilmad® NMR tube (5 mm diameter) and sealed using a polypropylene cap. This sample was used for all three types (^{1}H, ^{13}C and ^{19}F) of NMR. All NMR spectra were recorded at 25 °C on a 300 MHz Varian Mercury NMR spectrometer and processed using the Varian VNMRJ 3.2 software package. Referencing of

NMR spectra was carried out according to Gottlieb et al. (14) or IUPAC referencing with $CFCl_3$ set to 0 ppm.

2.3.5.1 ^{1}H NMR spectroscopy.

The spectrum was recorded at 300 MHz using 32 scans and was referenced to the signal of tetramethylsilane at 0 ppm.

2.3.5.2 ^{13}C APT NMR spectroscopy.

The spectrum was recorded at 75.4 MHz in Attached-Proton-Test mode using 22500 scans and was referenced to the central peak of the DMSO-d6 multiplet at 39.52 ppm.

2.2.5.3 ^{19}F NMR spectroscopy.

The spectrum was recorded at 282 MHz using 64 scans and was DMSO-d6 lock resonance frequency according to IUPAC referencing with $CFCl_3$ set to 0 ppm.

3. Results and discussion

As a first identification approach, a methanolic sample solution of the powder and herbal incenses were analyzed by GC-MS and major peaks were identified by means of computer based library search of the SWGDRUG Mass Spectral Library (Version 3.0) (15) and Cayman Spectral Library (Version 05202016) (16) installed on the Agilent ChemStation. The described HPLC-PDA method was applied as part of laboratories systematic identification

scheme of powders (ISO/IEC 17025:2005 accredited) using an in-house UV-spectrum database. No library hit was found. Also the search on Forendex, provided through the Southern Association of Forensic Scientists (17), did not result in the identification of the major compound. The analytical information obtained with the routine GC-MS and HPLC-PDA identification approach and the extended analysis by ESI-LC-MS, ESI-HRMS and NMR are summarized below.

3.1   GC-MS

As shown in Figure 2, a major peak at 26.5 min.was detected in the total ion chromatogram of the methanolic sample extracts of the herbal incenses. The retention time and mass spectrum was in accordance with the 5F-MDMB-PICA peak in the methanolic solution of the brown powder. The mass spectrum of 5F-MDMA-PICA and proposed fragmentation is given in Figure 2.

3.3   HPLC-PDA

As illustrated in Figure 3, the chromatographic profile obtained after injection of the methanolic sample solution of the powder and crude methanolic extract of the herbal incenses are similar. Besides the major peak at 28.24 min., two minor peaks (at retention time 30.06 min and 31.16 min, respectively) were detected with the same UV-spectrum as the major peak 5F-MDMB-PICA, indicating that two structure analogues are present. The UV-spectrum of the major peak is shown in Figure 3.

3.4   ESI-LC-MS

The LC-MS chromatogram indicates that material consists of one major component (retention time: 7.55 min) alongside several unidentified minor impurities. The ESI-LC-MS spectrum of the major component gives two peaks at 377.2 and 753.5 Da which could be assigned as the M+H and 2M+H peaks respectively. These values are consistent with earlier published literature on 5F-MDMB-PICA (18). Full LC-MS spectral data are shown in Figure 4.

3.5   ESI-HRMS and molecular formula analysis.

The high resolution spectrum for the sample gives two major peaks at 377.2250 and 753. 4460 ppm which could be assigned as the M+H and 2M+H ions respectively. Minor ions appearing in the spectrum could be attributed to M+Na (399.2083) and M+K ions (415.1834). Software analysis of the ion at 377.2250 Da pointed towards a molecular formula for the M+H ion $C_{12}H_{30}N_2O_3F$. These values are consistent with earlier published literature on 5F-MDMB-PICA (18). HRMS: [M+H] $C_{12}H_{30}N_2O_3F$ Calc: 377.2240 found: 377.2250. Full HRMS spectral data are shown in Figure 5 and 6.

3.6   NMR spectroscopy.

Figure 7 shows the recordings of the NMR spectra for ^1H, ^{13}C and ^{19}F, allowing the full assignment of all H, C and F atoms in the structure. Peak assignment can be correlated to the structure and atom numbering as shown in Figure 8. : ^1H NMR (300 MHz, DMSO-d6) δ = 8.42 (s, 1H, i), 8.12 (dd, J = 8.0 Hz, J = 1.0 Hz, 1H, n), 7.62 (d, J = 8.7 Hz, f), 7.54 (d, J = 8.0 Hz, 1H, k), 7.19 (dd, J = 8.0 Hz, J = 1.0 Hz , 1H, l), 7.15 (dd, J = 8.0 Hz, J = 1.0Hz, 1H, m),

7.53 Hz (s, 1H, c), 4.42 (dt, J_{HF} = 47.4 Hz, J = 6.0 Hz, 2H, t), 4.22 (t, J = 7.2 Hz, 2H, p), 3,67 (s, 3H, a), 1.90-1.80 (m, 2H, q), 1.77-1.59 (brm, 2H, s), 1.42-1.32 (m, 2H, r), 1.04 (s, 9H, e).

^{13}C NMR (75.4 MHz, DMSO-d6) δ = 172.0 (b), 164.4 (g), 136.1 (j), 131.7 (i), 126.7 (o), 122.0 (l), 121.2 (n), 120.7 (m), 110.3 (k), 108.7 (h), 83.6 (d, [1]J_{CF} = 162 Hz), 60.0 (c), 51.4 (a), 45.8 (p), 33.8 (d), 29.4 (d, [2]J_{CF} = 18.4 Hz, s) 29.3 (q), 26.8 (e), 22.2 (d, [3]J_{CF} = 5.8 Hz, r).

^{19}F NMR (282 MHz, DMSO-d6) δ = -217.0.

Apart from the principal compound both the ^{1}H and ^{13}C NMR spectra indicate the presence of a minor impurity having an ethyl substituent as indicated by the quartet at 3.05 ppm and the triplet at 1.2 ppm in the ^{1}H NMR spectrum and the presence of two peaks at 45 and 8 ppm in the ^{13}C NMR spectrum.

The full set of applied analytical methods allowed the identification and structural characterization of the major compound in the brown powder as a *tert*-leucinate synthetic cannabinoid: 2-[[1-(5-fluoropentyl)indole-3-carbonyl]amino]-3,3-dimethyl-butanoate or 5F-MDMB-PICA (synonyms 5F-MDMB-2201, MDMB-2201). Reported IUPAC names for this compound include: methyl 2-(1-(5-fluoropentyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate; methyl 2-{[1- (5-fluoropentyl)-1H-indol-3-yl]formamido}-3,3-dimethylbutanoate and N-[[1-(5-fluoropentyl)-1H-indol-3-yl]carbonyl]-3-methyl-L-valine, methyl ester).

All packages of "spice-like" herbal incenses were analyzed with GC-MS and HPLC-PDA. The presence of this synthetic cannabinoid was confirmed based on retention time and similarity index or mass spectrum.

5F-MDMB-PICA is structurally related to the previously identified synthetic cannabinoids 5F-MDMB-PINACA (synonyms: 5F-ADB, MDMB(N)-2201), differing by the replacement of the indazole core with an indole core (19-20). The chemical structure of *tert*-leucinate synthetic cannabinoids are shown in Figure 9 (18).

A PubMed search demonstrates the lack of information in the NPS field: only a single reference whatsoever was found for the substance. Banister et al. (18) studied the pharmacology of valinate and *tert*-leucinate synthetic cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINICA, MDMB-FUBINICA, MDMB-CHMICA and their analogues.

In October 2016, the Joint Research Centre, the European Commission's in house science service, reported the analysis of a pink-orange powder received from the Belgian Customs laboratory, confirmed to be 5F-MDMB-PICA (21).

4. Conclusions

The full set of applied analytical methods allowed the identification and structural characterization of the synthetic cannabinoid methyl 2-[[1-(5-fluoropentyl)indole-3-carbonyl]amino]-3,3-dimethyl-butanoate or 5F-MDMB-PICA in powder and as additive in "spice-like" herbal incenses.

References

1.   Publications Office of the European Union. European Drug Report, Trends and Development. European Union Publications [Internet]. 2016 May 1; emcdda.europa.eu/system/files/publications/2637/TDAT16001ENN.pdf (Accessed 10/12/2016).

2.   Blanckaert, d. P. (2016). Belgian Early Warning System Drugs, personal communication. Brussels: Scientific Institute of Public Health, Belgium.

3.   European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). New psychoactive substances in Europe-Legislation and prosecution-current challenges and solutions (2016), emcdda.europa.eu/system/files/publications/3353/TD0416736ENN.pdf (Accessed 15/11/2016)

4.   P. Adamowicz, J. Gieroń, D. Gil, W. Lechowicz, A. Skulska, B. Tokarczyk. The prevalence of new psychoactive substances in biological material - a three-year review of casework in Poland. Drug Test Anal, 8(1): 63–70 (2016).

5.   S. Mörkl, C. Blesl, W. E. Wurm, A. Tmava. Acute Psychosis after Consumption of Synthetic Cannabinoids. Fortschr Neurol Psychiatr, 84(3): 150–154 (2016).

6.   P. Kabata, D. Schetz, W. Waldman, M. Wiergowsk, J. Sein Anand. Analysis of intoxication with novel psychoactive substance (NPS) in Pomeranian region, from January to July 2015. Prz Lek, 72(10): 517–521 (2015).

7.   P. Adamowicz. Fatal intoxication with synthetic cannabinoid MDMB-CHMICA. Forensic Sci Int, 261: e5–10 (2016).

8. S.L. Hill, J. Najafi, M. Dunn, P. Acheampong, A. Kamour, J. Grundlingh, P.G. Blain, S.H. Thomas. Clinical toxicity following analytically confirmed use of the synthetic cannabinoid receptor agonist MDMB-CHMICA. A report from the Identification Of Novel psychoActive substances (IONA) study. Clin Toxicol (Phila), 54(8): 638–643 (2016).

9. A. Seywright, H.J. Torrance, F.M. Wylie, D.A. McKeown, D.J. Lowe, R. Stevenson Analysis and clinical findings of cases positive for the novel synthetic cannabinoid receptor agonist MDMB-CHMICA. Clin Toxicol (Phila), 54(8): 632–637 (2016).

10. A.A. Westin, J. Frost, W.R. Brede, P.O.M Gundersen, S. Einvik, H. Aarset, L. Slordal. Sudden Cardiac Death Following Use of the Synthetic Cannabinoid MDMB-CHMICA. J Anal Toxicol, 40(1): 86-7 (2015).

11. N. Langer, R. Lindigkeit, H.-M. Schiebel, U. Papke, L. Ernst, T. Beuerle. Identification and quantification of synthetic cannabinoids in "spice-like" herbal mixtures: update of the German situation for the spring of 2015. Forensic Toxicol, 34(1): 94–107 (2016).

12. European Monitoring Centre for Drugs and Drug Addiction (2016), EMCDDA–Europol Joint Report on a new psychoactive substance: methyl 2-[[1-(cyclohexylmethyl)indole-3-carbonyl]amino]-3,3-dimethylbutanoate (MDMB-CHMICA), Joint Reports, Publications Office of the European Union, Luxembourg. emcdda.europa.eu/system/files/publications/2873/2016.4528_WEB.pdf (Accessed 10/12/2016).

13. Commision E. Proposal for a COUNCIL DECISION on subjecting the new psychoactive substance methyl 2-[[1-(cyclohexylmethyl)-1Hindole-3-carbonyl]amino]-

3,3-dimethylbutanoate (MDMB-CHMICA) to control measures. EC EU publications [Internet]. 31 Aug 2016.

eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52016PC0548 (Accessed 10/12/2016).

14. H.E. Gottlieb, V. Kotlyar, A. Nudelman. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. J Org Chem, 62 (21), 7512–7515 (1997).

15. Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG). SWGDRUG mass spectral library. http://www.swgdrug.org/ms.htm. (Accessed 22/07/2016).

16. Cayman Chemical. Cayman spectral library. https//www.caymanchem.com/app/ template/SpectralLibrary.vm. (Accessed 01/06/2016).

17. Southern Association of Forensic Scientists. forendex.southernforensic.org. (Accessed 13/11/2016).

18. S.D. Banister, M. Longworth, R. Kevin, S. Sachdev, M. Santiago, J. Stuart, J.B.C Mack, M. Glass, I.S. McGregor, M. Conner, M. Kassiou. Pharmacology of Valinate and *tert*-Leucinate Synthetic Cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and Their Analogues. ACS Chem Neurosci, 7(9): 1241–1254 (2016).

19. V. Shevyrin, V. Melkozerov, A. Nevero, O. Eltsov, Y. Shafran, Y. Morzherin, A.T. Lebedev. Identification and analytical characteristics of synthetic cannabinoids with an indazole-3-carboxamide structure bearing a *N*-1-methoxycarbonylalkyl group. Anal Bioanal Chem, 407 (21): 6301–6315 (2015).

20. K. Hasegawa, A. Wurita, K. Minakata, K. Gonmori, I. Yamagishi, H. Nozawa, K. Watanabe, O. Suzuki. Identification and quantitation of 5-fluoro-ADB, one of the most dangerous synthetic cannabinoids, in the stomach contents and solid tissues of a human cadaver and in some herbal products. Forensic Toxicol, 33: 112-121 (2015).

21. Joint Research centre (2016).

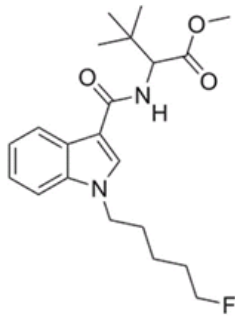http://www.policija.si/apps/nfl_response_web/0_Analytical_Reports_final/5F-MDMB-PICA-ID-JRC16100003rpt.pdf (Assessed 21/01/2017).

Figure 1. Chemical structure of 5F-MDMB-PICA.
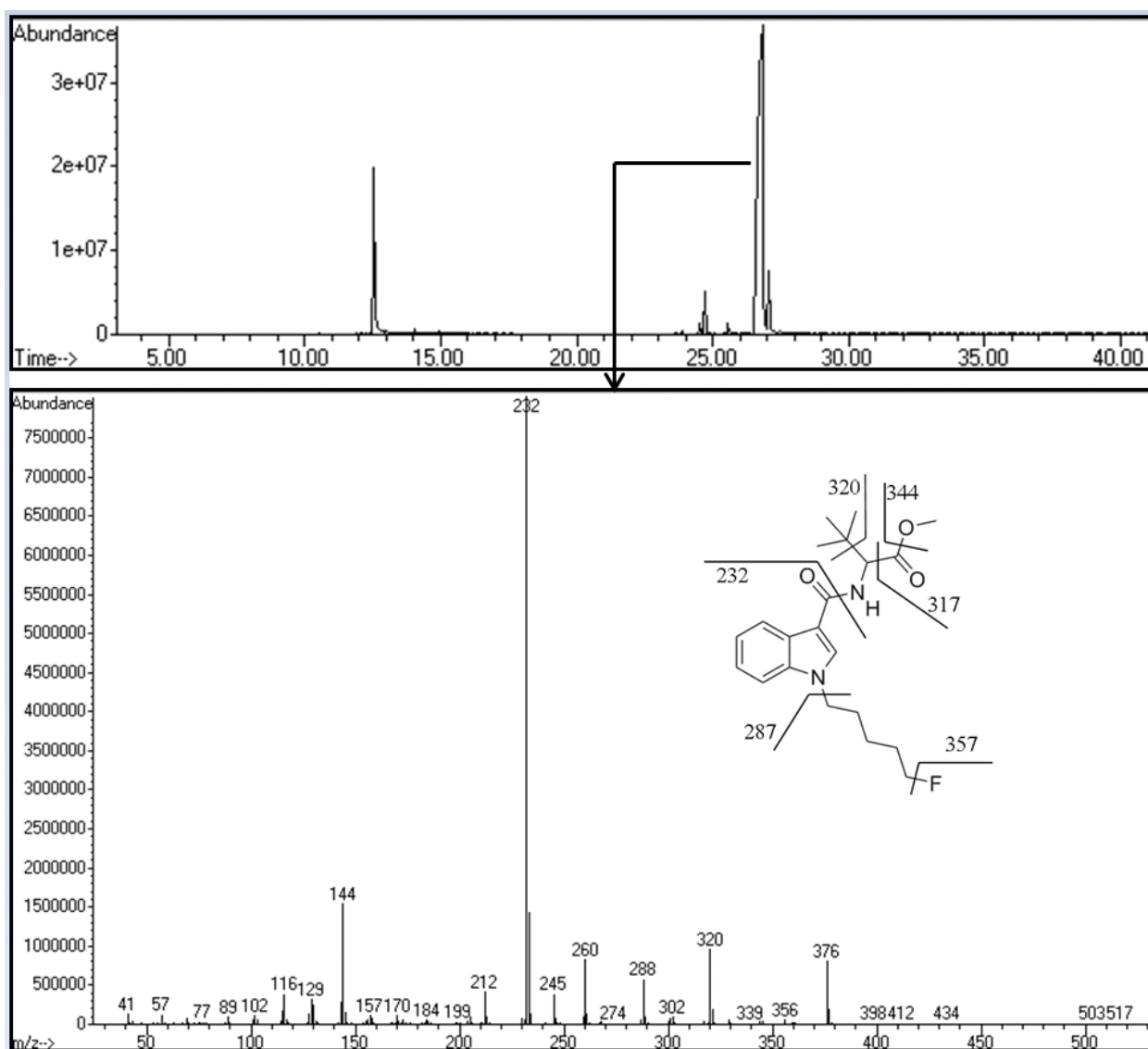
Figure 2. GC-EI-MS total ion chromatogram (diphenylamine: peak at retention time 12.6 min) of a herbal incense (upper): mass spectrum of the major compound 5F-MDMB-PICA at retention time 26.5 min with proposed fragmentation (lower).
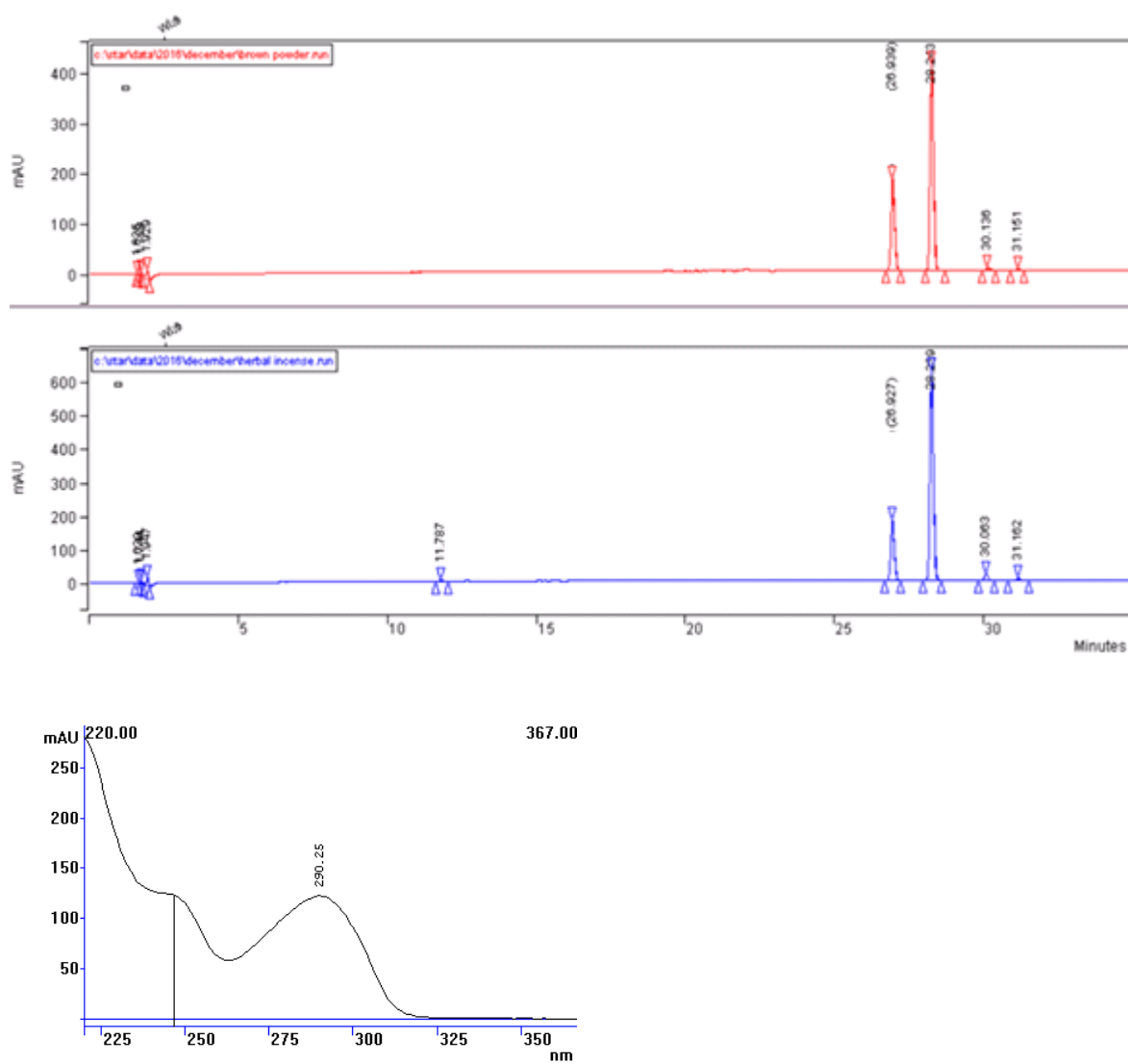
Figure 3. HPLC-PDA-analysis (diphenylamine: peak at retention time 26.93 min): chromatogram recorded at 254 nm of the brown powder (upper) and a herbal incense (middle), with UV-spectrum of 5F-MDMB-PICA at retention time 28.24 min (lower).
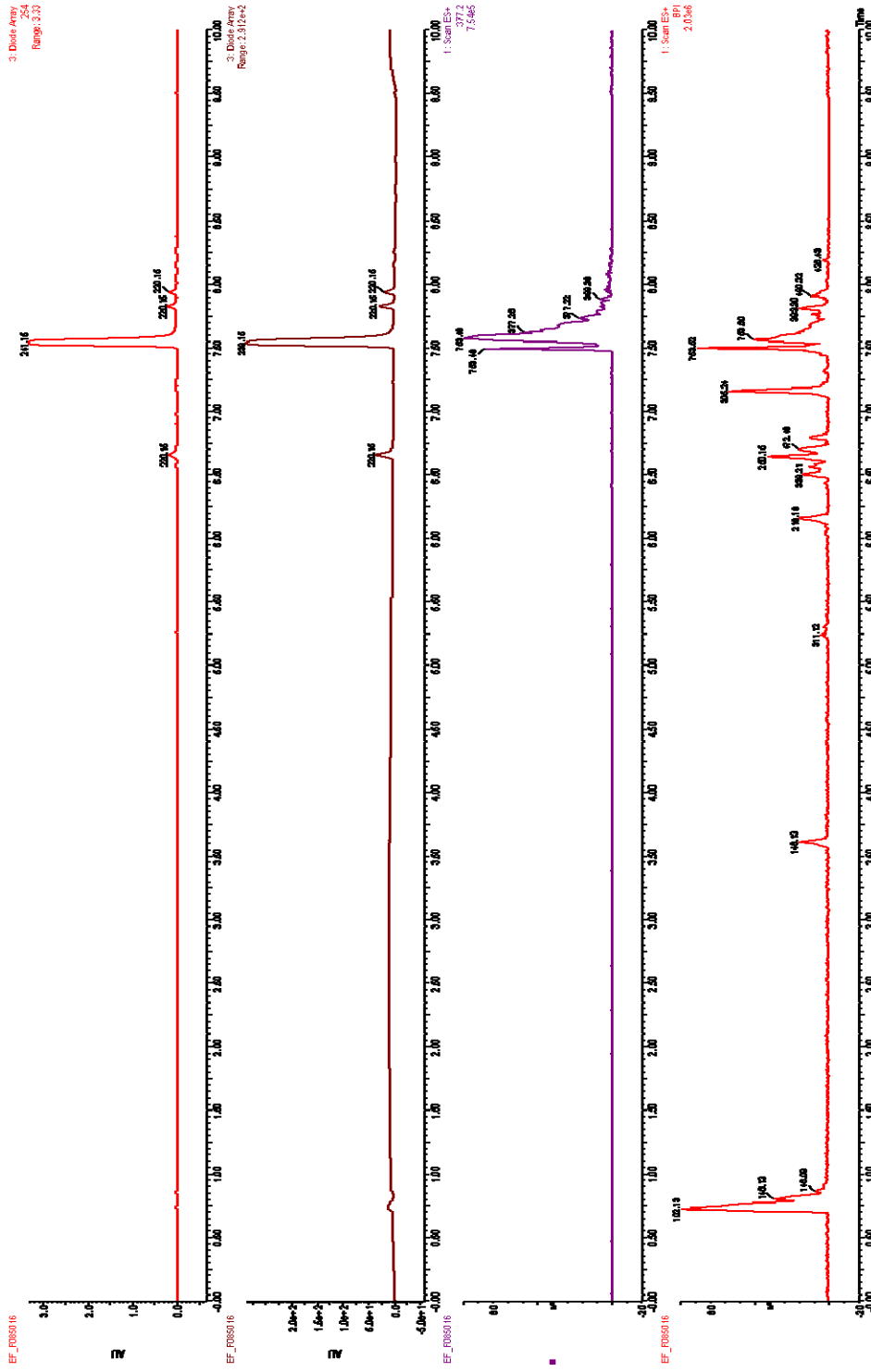
Figure 4. LC-MS chromatogram of 5F-MDMB-PICA.
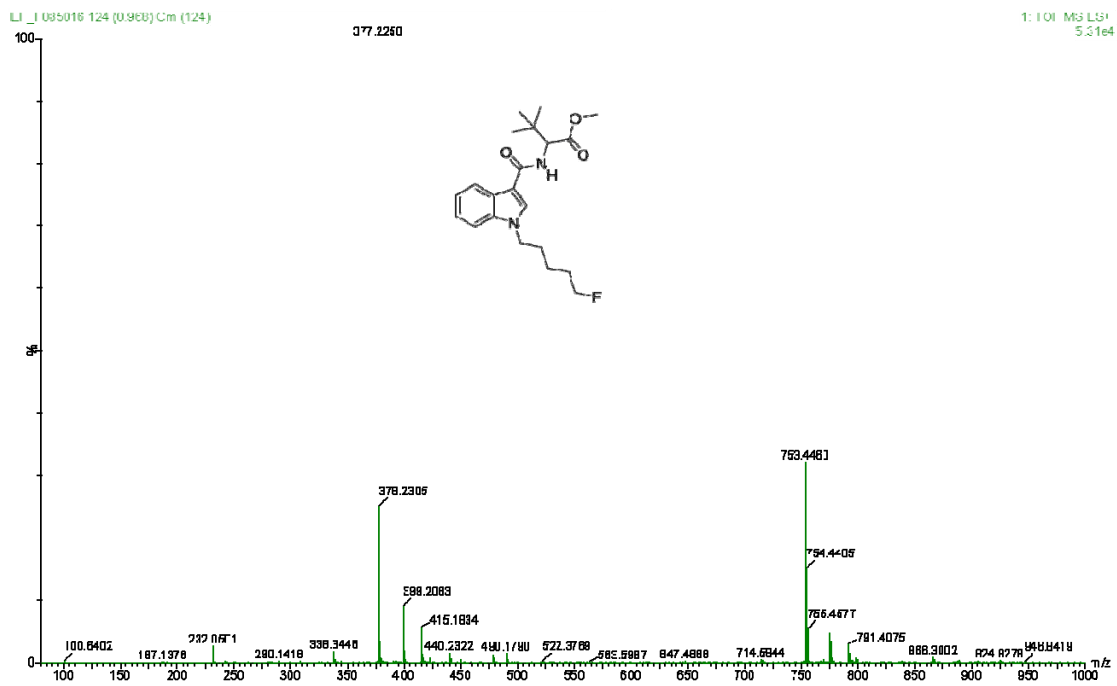
Figure 5. HRMS spectrum of 5F-MDMB-PICA

Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
656 formula(e) evaluated with 6 results within limits (up to 50 best isotopic matches for each mass)

Elements Used: C: 0-60   H: 0-100   N: 1-5   O: 1-5   F: 0-6

| | | | EF_F085016 124 (0.968) Cm (124) | 16-Nov-2016 | brown powder |
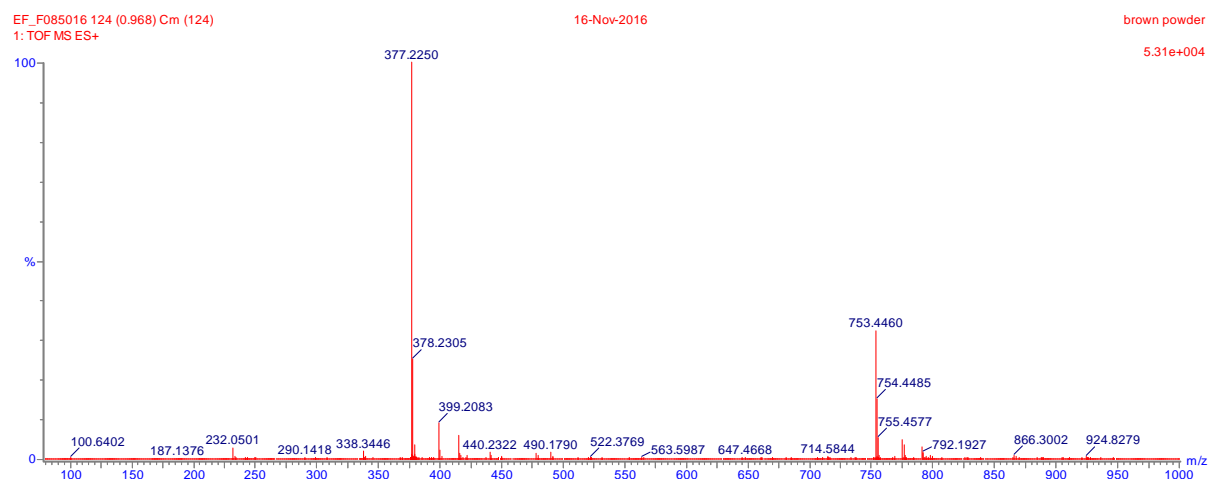
Minimum: -1.5
Maximum: 5.0   10.0   50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
377.2250	377.2240	1.0	2.7	7.5	377.9	0.0	C21 H30 N2 O3 F
	377.2229	2.1	5.6	11.5	385.2	7.3	C24 H29 N2 O2
	377.2252	-0.2	-0.5	3.5	385.8	7.8	C18 H31 N2 O4 F2
	377.2216	3.4	9.0	4.5	386.1	8.2	C19 H29 N2 O F4
	377.2263	-1.3	-3.4	0.5	388.5	10.6	C15 H32 N2 O5 F3
	377.2227	2.3	6.1	0.5	388.6	10.7	C16 H30 N2 O2 F5

Figure 6. HRMS based Molecular ion analysis
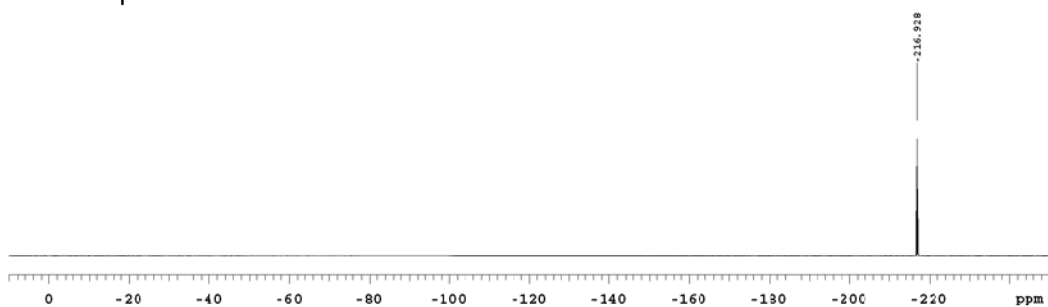
A: ¹H-spectrum
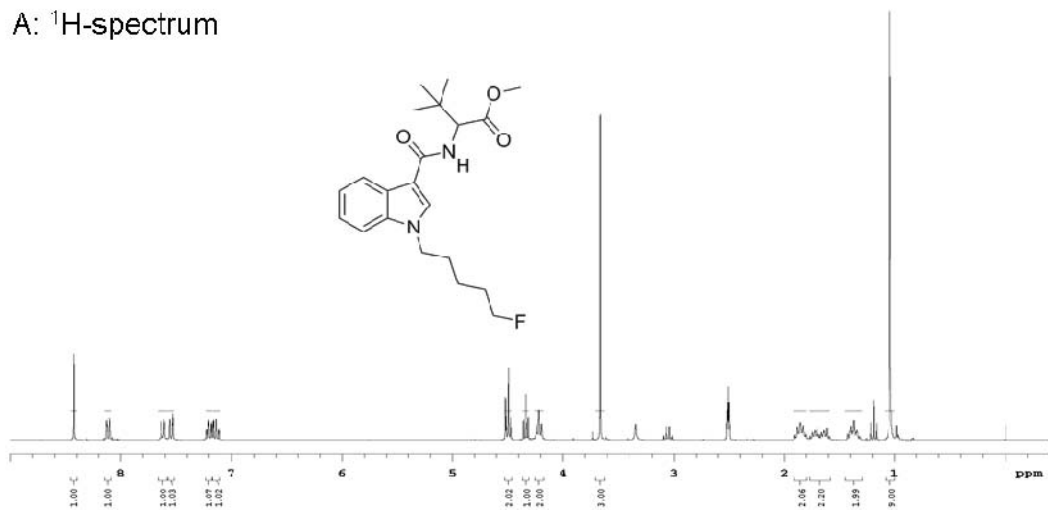
B: ¹³C-spectrum
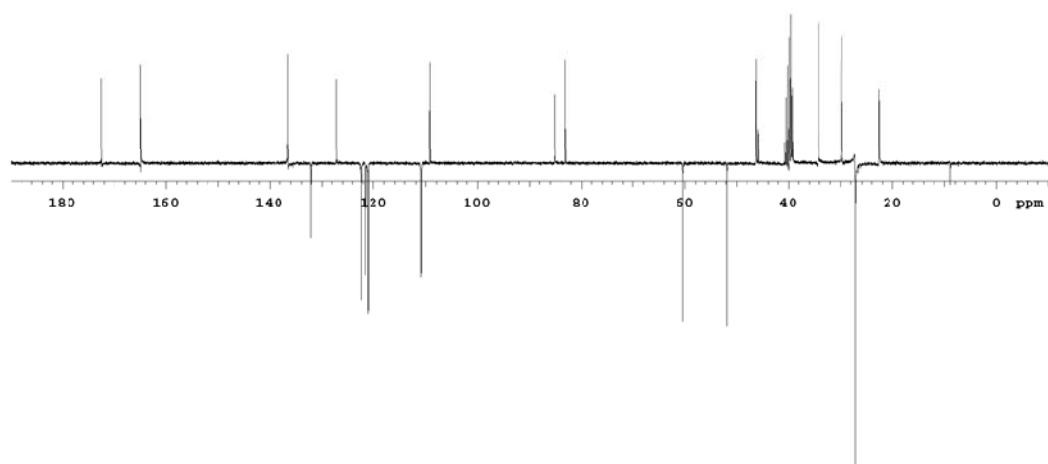
C: ¹⁹F-spectrum

Figure 7. ¹H-,¹³C-and ¹⁹F-NMR spectra of 5F-MDMB-PICA.
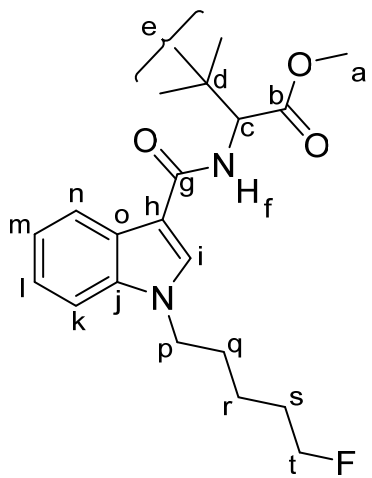
Figure 8. Atomic numbering in 5F-MDMB-PICA used for NMR peak assignment.

R = CH₃, X = CH : 5F-MDMB-PICA
R = H,   X = CH : 5F-AMBICA
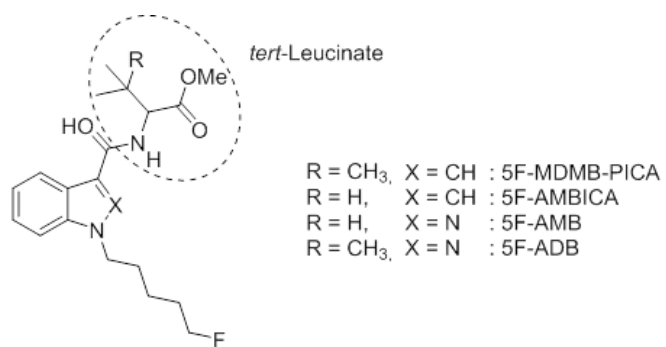R = H,   X = N : 5F-AMB
R = CH₃, X = N : 5F-ADB

Figure 9. Chemical structure of *tert*-leucinate synthetic cannabinoids