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The isolation and characterisation of the synthetic cannabinoid AM-2201 from commercial products using purification by HPLC-DAD

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Abstract: A total of six products containing legal highs were purchased via the internet from the UK- based retailers and screened for the presence of synthetic cannabinoids using a fast GC-MS method and identified, in the absence of reference materials, by comparing the mass spectra with the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) mass spectral library. Four synthetic cannabinoids were detected: RCS-4, CP-47, 497, UR-144 and AM-2201. The active ingredient (1-(5-fluoropentyl)-3-(1-naphthoyl) indole), with the street name AM-2201, detected in the product named Doob was isolated and purified from the methanolic extract of the product using preparative HPLC with analytical column (column overloading method). The structure of the substance was confirmed using NMR. This approach used common analytical equipment found in forensic and other analytical laboratories (except for the NMR), therefore can be useful for the identification of unknown psychoactive substances in drugs of abuse.

Keywords: AM-2201, fast GC-MS, preparative HPLC

1 Introduction

Synthetic cannabinoid receptor agonists, also known as synthetic cannabinoids, are a class of novel psychoactive substances (NPS), found in herbal and incense products. In the few years since their first use as novel psychoactive substances the number of chemically different synthetic cannabinoids on the market exceeds 100. The EU Early warning system, which provides information about new drugs to EU member states, identified in 2012 as many as thirty new synthetic cannabinoid receptor agonists on the market, i.e. 41% of the total number of newly reported drugs (EMCDDA, 2013). The appearance of new substances on the market is related to the current local laws. Authors who have investigated the phenomenon of synthetic cannabinoids agree that, if a substance is put under control, new ones not recognized by law will appear on the market (Dargan et al. 2011; Fattore & Fratta 2011; Hudson & Ramsey 2011) and continuous surveillance of the drug market is necessary.

There are certain problems regarding analysis of synthetic cannabinoids that analytical laboratories are facing: often very low and variable concentration of synthetic cannabinoids products (Zuba et al. 2011, Choi et al., 2013), the presence of masking substances (tocopherol, esters of fatty acids, oleamide), scents, aromas and natural components from the plants used as carriers in products are the problems related to the clandestine manufacturing process of the products containing them. The dynamicity of the drug market creates a problem that many forensic laboratories are facing: the lack of reference materials.

As new products appear regularly on the market, the industry cannot keep pace with clandestine laboratories regarding analytical tests and reference material for new substances: often laboratory standards are not easily available, or they are expensive (Denooz et al. 2013; Vardakou et al. 2010; Zuba et al. 2011); furthermore, many substances have structural isomers which makes analysis even more demanding, since the standards of possible isomers should be analysed before a definitive conclusion about a specific isomer can be made. As long as some of these substances are legal, they probably will not be routinely analysed so manufacturers will not have interest in producing standards, which may also increase the price of available ones.

The isolation of the active principle from the analysed product has been used to obtain the pure substance, that can be subjected to NMR spectroscopy

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for definitive proof of the structure (Denooz et al. 2013; Ernst et al. 2011; Ernst et al. 2012; Moosmann et al. 2012; Jankovics et al. 2012). The isolation was done using silica gel column chromatography (Ernst et al., 2012; Ernst et al., 2011), flash chromatography (Moosmann et al., 2012) and open column chromatography (Choi et al., 2012). With the increase in number of new substances appearing on the market, there is a demand for easily available reference material that can be obtained by isolation from the product. To use the commonly found analytical equipment for the isolation would be an advantage, since most forensic laboratories could isolate the active principle within a few days.

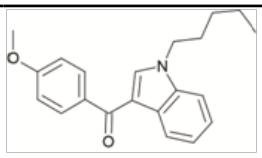
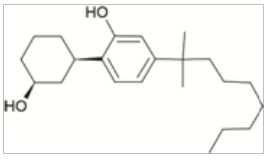
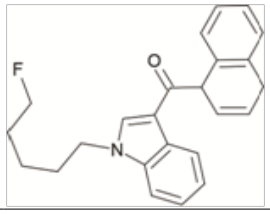
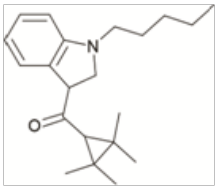
Here we propose a laboratory protocol that uses standard laboratory instrumentation firstly, to screen samples for potential NPS and then to purify and confirm the identity of the potential NPS. To demonstrate the protocol six herbal products were purchased from UK-based web sites and screened for the presence of synthetic cannabinoids using a fast GC-MS method; a modification of a method previously developed in our laboratory for the analysis of novel psychoactive substances (Elie et al., 2012). The method successfully separates a range of synthetic cannabinoids that are tentatively identified by mass spectral comparison with the SWGDRUG mass spectral library. As a proof of principle the synthetic cannabinoid AM-2201 was selected for purification using an analytical HPLC system in a preparative mode. The purity of AM 2201 was confirmed by HPLC-DAD and its chemical structure confirmed using NMR.

2 Materials and methods

2.1 Samples

Six herbal products were purchased from UK-based web sites (Table 1). Samples 1 and 5 had the same brand name

Table 1: Synthetic cannabinoids found in samples 1-6, along with retention times and formulas:

Sample Number	Product Name/Date Purchased	Identified Synthetic cannabinoid(s)	Retention time (min)	Chemical Structure
1	Pineapple Express July 2012	RCS-4	2.29	
2	Spice Diamond	(+/-) CP 47,497- C8 Homolog	1.49	
3	DOOB December 2012	AM-2201	3.80	
4	UR-144 December 2012	UR-144 AM-2201	1.20 3.78	
5	Pineapple Express December 2012	RCS-4	2.30	
6	Doob December 2012	AM-2201	3.85	

(Pineapple express) and the same packaging but were purchased from different retailers and with a 5 month difference in purchase date. Samples 3 and 6 had the same name (Doob) but were purchased from different retailers and with different packaging. The product name of sample 4 (UR-144) suggested the active principle from the sample was UR-144. Sample 2 was obtained prior to the generic control of synthetic cannabinoids in the UK in December 2009.

2.2 Preparation of sample extracts

The full content of each package was homogenised using a previously reported technique using medium grade (100 grit) sand paper (Logan et al. 2012). Homogenization was done by rubbing the herbal material between two pieces of sand paper (10 x 10cm each). A piece of sand paper was used in the later GC-MS analysis as a negative control.

100 mg of ground herbal material was extracted using 1ml of methanol (HPLC grade, Fischer Scientific, UK), followed by vortexing for 5 minutes and sonication for 10 minutes. The extracts were filtered through 0.45µm syringe filters (Chromacol) into vials and frozen at -20 °C until use.

All the analyses were carried out using the methanolic extracts, except for the NMR analysis.

2.3 Analytical Conditions

Analytical and preparative HPLC analyses were done using Merck Hitachi HPLC system with C_{18} column (Eclipse Plus C18, 4.6 x 250mm, 5µm, Agilent) and diode array detector L-7455. The mobile phase used was methanol:water (70:30), and the analytes were separated under isocratic conditions. The mobile phase flow was 1 ml/min, and the oven temperature was 40°C. The scan range of the diode array detector (DAD) was 200-400 nm. In the analytical mode, the injection volume of the extracts was 10µl and the run time 45 minutes, while for the isolation of the substance (preparative mode) 40 µl of sample 6 extract was repeatedly injected using a column overload procedure.

The extracts of the 6 samples were screened using a fast GC-MS method (Elie et al, 2013) modified for synthetic cannabinoid analysis. Analysis was carried out using a Perkin Elmer Clarus 600 gas chromatograph with column SLB™-5ms fused silica capillary column (10m x 0.1mm x 0.1µm film thickness) (Supelco analytical). The gas chromatograph was coupled with Perkin Elmer Clarus 600C mass spectrometer, equipped with Perkin Elmer TurboMass software (2008).

The initial conditions for the gas chromatograph and the mass spectrometer were set according to data from the literature for the conventional GC-MS methods for synthetic cannabinoids (Zuba et al. 2011) and the method for screening for other legal highs currently used in the laboratory (Elie et al., 2012), since no methods have been optimized for synthetic cannabinoids using fast GC-MS. The oven programme was initial temperature 240°C, then ramp to 290°C at rate 50°C/min, ending with a plateau for 6 minutes (total run time 7 minutes). Mass spectra were searched against NIST and SWGDRUG libraries NIST compatible, in demo version of NIST MS search 2.0 programme.

The ^{1}H NMR analysis of the isolated substance was performed in Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University. The instrument used was multi nuclear gradient Bruker Avance 500 MHz. The solvent used for the NMR analysis was deuterium labelled chloroform ($CDCl_3$) (supplier).

2.4 Determination of the HPLC retention time of the target substance

The eluent corresponding to each HPLC peak of a 20 µl injection of Sample 6 extract was collected as a separate fraction using a simple manual procedure. This was carried out in triplicate and the fractions for each peak were pooled and evaporated to dryness under a gentle stream of nitrogen, reconstituted in 75 µl methanol and analysed by the fast GC-MS method.

2.5 Isolation of the target substance

A total volume of 640 µl of sample 6 extract (16 x 40 µl injections) was injected and the fraction containing the target substance was collected. The 16 separate fractions were pooled and the mobile phase was evaporated under a stream of nitrogen. When almost fully evaporated the solution was transferred to a 1.5 ml eppendorf tube and evaporated to dryness. 0.5 mg of the isolated substance was dissolved in methanol and analysed by HPLC as a purity check. Ratios of the isolated substance's peak area and total peak area of the chromatogram were used to calculate purity of the substance. 2.7 mg of the isolated substance was analysed by NMR.

3 Results and Discussion

Fast GC-MS analysis, using MS comparison with the SWGDRUG mass spectral library, suggested the presence

of four synthetic cannabinoids in the purchased samples (Table 1). Fast GC-MS of methanolic extracts provides a simple and effective tool that we have applied in our laboratory for screening large numbers of drug samples in NPS casework. When coupled with the SWGDRUG library and NIST software it provides a tentative identification even when specific drugs may not have been previously analysed in our laboratory. The fast GC-MS screening method was initially developed for detection of amphetamine/cathinone based NPS (Elie et al, 2012). A number of the more recently available synthetic cannabinoid NPS do not elute under these conditions and so the GC programme was modified to run at higher temperatures with a longer runtime when synthetic cannabinoids are suspected in the sample such as in the analysis of herbal NPS products. This still provides a fast screening method as shown by the retention times of all the identified synthetic cannabinoids in table 1 being less than 4 minutes.

As a proof of principle one of the Doob samples (sample 6) was selected for purification of the NPS component by HPLC. The peak at 3.85 minutes in the chromatogram of Sample 6 (figure 1) was identified as AM-2201, a synthetic cannabinoid first synthesized by Alexandros Makriyannis (Makriyannis et al, 2007).

Analytical HPLC of sample 6 extract showed six peaks in the chromatogram (figure 2). Each peak of the extract was isolated, collected and analysed by the fast GC-MS method. This identified the HPLC peak with retention time 16.67 minutes as being the substance identified as AM-2201. The other peaks could not be matched against the SWGDRUG database in agreement with the fast GC-MS analysis of the methanol extract (figure 1).

This substance was subsequently isolated from 640 µl of sample 6 extract using a column overload preparative mode of HPLC using the same analytical column. A total mass of 3.2 mg of the substance was isolated using a total volume of 1667 ml methanol and a total run time of 2035 minutes that included blank runs between each extract run. From the mass of AM-2201 recovered and the volume of extract analysed, the calculated concentration in sample 6 was 5.2% (52 mg/g product) assuming that the recovery was 100%.

The HPLC chromatogram (figure 3) confirms the purity of the isolated substance and provides an estimate of purity of 97.5% calculated using the ratio of the peak area at 16.67 minutes to the total area under the chromatogram. The DAD UV spectrum (figure 3) is also shown with main absorption bands at 220 and 315 nm which is in agreement with the spectrum previously reported (Denooz et al, 2013).

The remaining isolated substance was analysed by both ^{1}H and ^{13}C NMR. The spectral data are presented in table 2 and both proton and carbon assignments are in agreement with the molecular formula of $C_{24}H_{22}NOF$ and with the data presented by Denooz et al confirming the substance as AM-2201. This agreement also confirms the structure as the 1-naphthyl isomer and not the 2-naphthyl isomer. AM-2201 is an example of one of the group of second generation synthetic cannabinoids that appeared on the legal high market following the UK generic control of synthetic cannabinoids in 2009. It belongs to the class known as naphthoylindoles and circumvents the 2009 generic control by having a fluoro-substituent on the alkyl side-chain. In early 2013 AM-2201 was one of a number of second generation synthetic cannabinoids to be controlled in the UK.

Application of a column overload method by injecting an increased volume of the sample, as a preparative HPLC method, means that smaller amount of the isolated substance can be obtained and the time for the isolation will be longer as compared to the application of a preparative column. However, this approach can be used in more laboratories since preparative columns are not part of routine analytical equipment. Obtained UV spectra can be used as additional information about the substance analysed and help with the identification if there are spectral libraries available.

4 Conclusions

With the frequency at which new synthetic cannabinoid receptor agonists appear on the legal highs market, it is difficult for analytical laboratories to have all possible reference materials needed for the unequivocal identification of the present drugs. In the absence of reference materials isolation of the analysed substance from the mixture is an alternative approach since the NMR analysis of the isolated and purified substance can provide the definitive identification. Several milligrams of the substance are sufficient for NMR analysis and with isolation of larger amounts it can be used as an in-house purified reference material for further analyses. The isolation approach using a column overload method with analytical HPLC column is suitable for use in most analytical laboratories.

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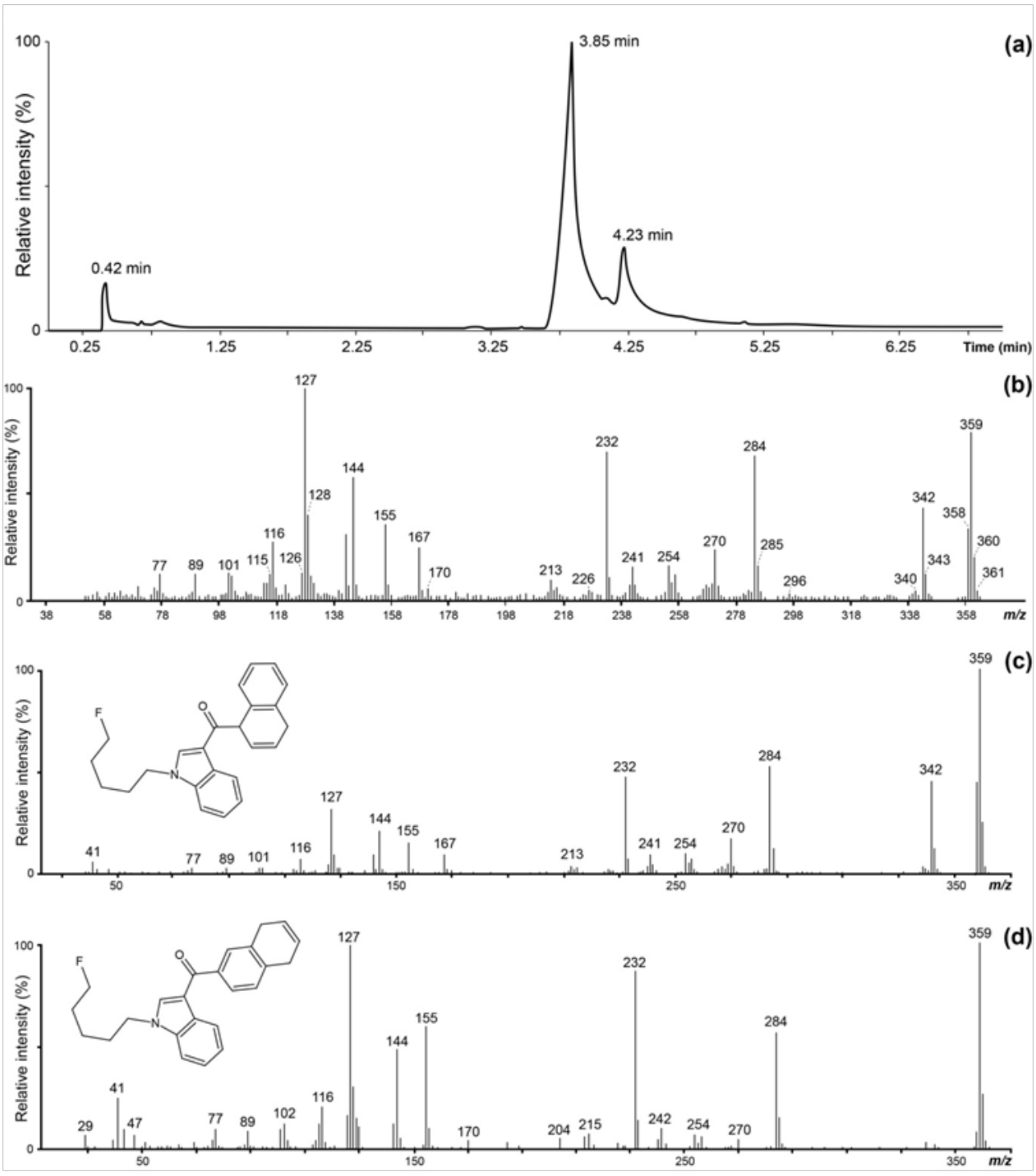


Figure 1: (a) Fast GC-MS chromatogram of the sample 6 extract and (b) EI mass spectrum of the peak at 3.85 minutes. The mass spectra of AM-2201 and its 2' naphthyl isomer taken from the SWGDRUG 2013 MS library are also shown as spectra (c) and (d) respectively.

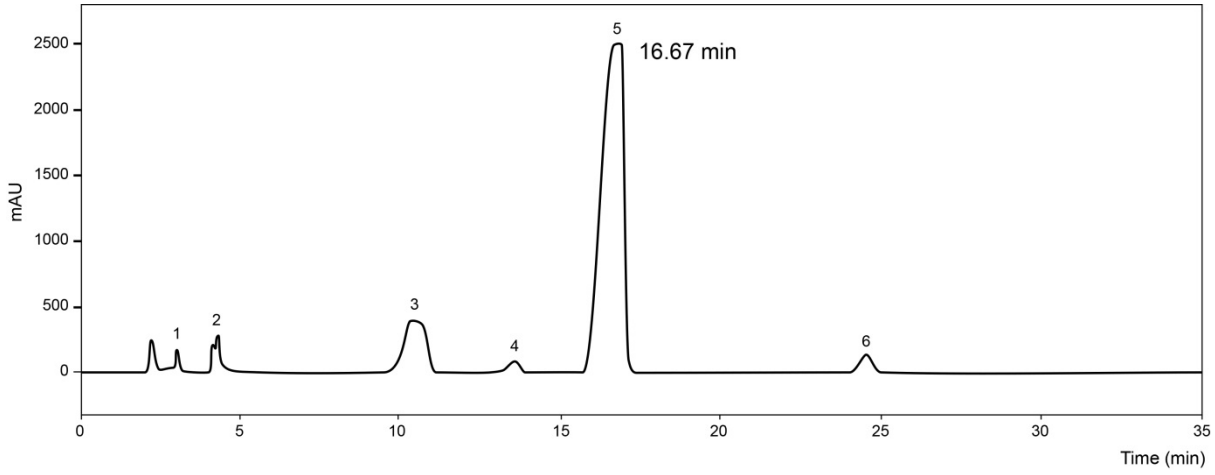


Figure 2: Sample 6 extract HPLC chromatogram with the six peaks that were isolated and ran on the fast GC-MS to determine the retention time of the peak containing AM-2201.

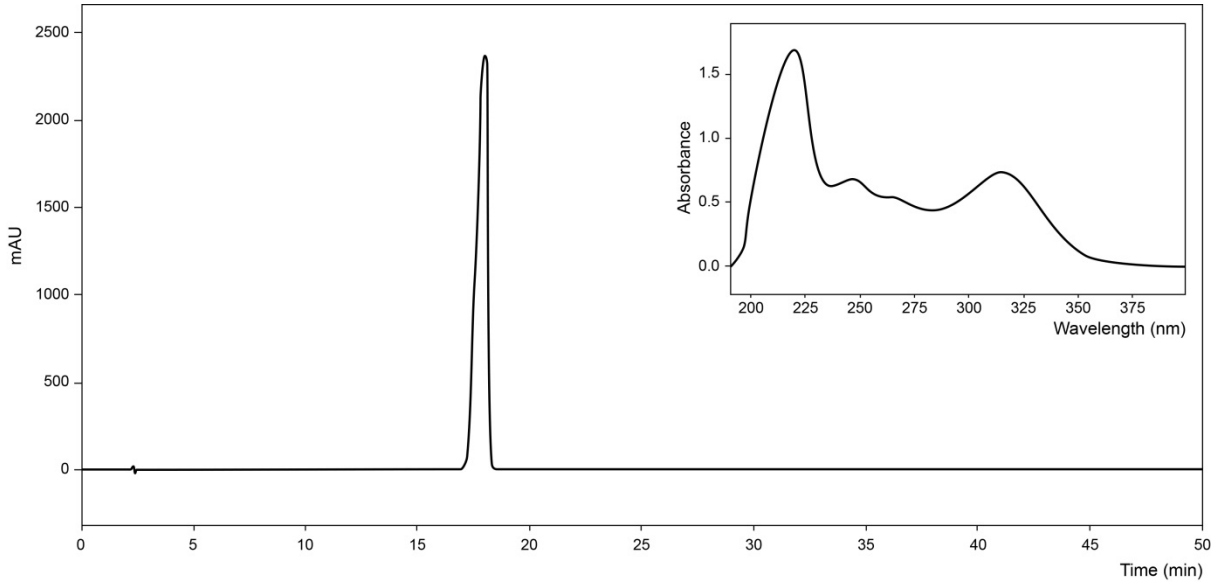
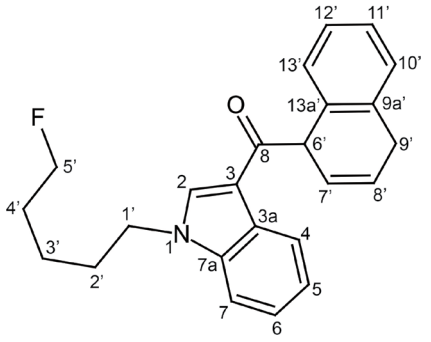


Figure 3: HPLC chromatogram of the isolated substance with its Diode Array Detector UV spectrum (insert).

Table 2: ^{1}H and ^{13}C NMR spectral data of the isolated substance (AM-2201), recorded at 500 MHz in $CDCl_3$. Chemical shifts (δ) and multiplicities are presented in the table, except for the aromatic hydrogens in positions 4, 5, 6, 7', 8', 11' and 12', the signals of which could not be fully resolved. Comparison is made with the data of Denooz et al.



Position	1H* (δ [ppm], multiplicities**, the number of hydrogens)	Denooz et al	13C	Denooz et al
1	-	-	-	-
1'	4.10 (t, 2H)	4.28 (t, 2H)	47.81	46.17 (CH_2)
2'	1.90 (m, 2H)	1.84 (m, 2H)	30.49	28.99 (CH_2)
3'	1.45 (m, 2H)	1.28 (m, 2H)	23.60	21.90 (CH_2)
4'a	1.65 (m, 1H)	1.58 (m, 1H)	30.88	29.13 (CH_2)
4'b	1.75 (m, 1H)	1.61 (m, 1H)	31.04	29.28 (CH_2)
5'a	4.35 (t, 1H)	4.31 (t, 1H)	83.94	82.93 (CH_2)
5'b	4.45 (t, 1H)	4.41 (t, 1H)	85.25	84.22 (CH_2)
2	7.70 (s, 1H)	7.79 (s, 1H)	141.01	139.36 (CH)
3	-	-	118.30	116.08 (C)
3a	-	-	138.75	136.79 (C)
4	7.50-7.60**	7.64(m, 1H)	111.70	111.03 (CH)
5	7.35-7.40*	7.33 (m, 1H)	124.87	123.30 (CH)
6	7.35-7.40*	7.33 (m,1H)	124.01	122.46 (CH)
7	8.50 (d, 1H)	8.32 (d, 1H)	123.39	121.71 (CH)
7a	-	-	128.21	126.38 (C)
8	-	-	194.73	190.86 (C)
6'	-	-	140.19	139.36 (C)
7'	7.50-7.60**	7.65 (m, 1H)	126.55	125.70 (CH)
8'	7.50-7.60**	7.64 (m, 1H)	125.84	124.96 (CH)
9'	8.20 (d, 1H)	8.09 (d, 1H)	131.20	129.68 (CH)
9'a	-	-	135.29	133.31 (C)
10'	8.00 (d, 1H)	8.03 (d, 1H)	129.45	128.27 (CH)
11'	7.35-7.40*	7.56 (t, 1H)	127.44	126.26 (CH)
12'	7.35-7.40*	7.51 (t, 1H)	127.85	126.70 (CH)
13'	7.95 (d, 1H)	8.01 (t, 1H)	126.98	125.27 (CH)
13a'	-	-	132.03	130.06 (C)

* a group of 4H not fully resolved; ** a group of 3H not fully resolved

References

Choi H., Heo S., Kim E., Hwang B.Y, Lee C., Lee J., Identification of (1-pentylindol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone and its 5-pentyl fluorinated analog in herbal incense seized for drug trafficking, Forensic Toxicology, 2013, 31 (1), 86-92.

Dargan P.I., Hudson S., Ramsey J., Wood D. M., The impact of changes in UK classification of the synthetic cannabinoid receptor agonists in "Spice", The International Journal on Drug Policy, 2011, 22(4), 274–7.

Denooz R., Vanheugen J. C., Frederich M., de Tullio P., Charlier C., Identification and structural elucidation of four cannabimimetic compounds (RCS-4, AM-2201, JWH-203 and JWH-210) in seized products, Journal of Analytical Toxicology, 2013, 37(2), 56–63.

Elie M.P., Elie L.E., Baron M.G., Keeping pace with NPS releases: fast GC-MS screening of legal high products, Drug Testing and Analysis, 2012, 5(5), 281-290.

Ernst L., Schiebel H. M., Theuring C., Lindigkeit R., Beuerle T., Identification and characterization of JWH-122 used as new ingredient in "Spice-like" herbal incenses, Forensic Science International, 2011, 208(1-3), e31–5.

Ernst L., Kruger K., Lindigkeit R., Schiebel H. M., Beuerle T., Synthetic cannabinoids in "spice-like" herbal blends: first appearance of JWH-307 and recurrence of JWH-018 on the German market, Forensic Science International, 2012, 222(1-3), 216–22

European Monitoring Centre for Drugs and Drug Addiction-EMCDDA. European Drug Report. Trends and developments 2013. [pdf] Available at http://www.emcdda.europa.eu/publications/edr/trends-developments/2013 , Acessed on 30.06.2013. doi:10.2810/88175

Fattore L., Fratta W., Beyond THC: The New Generation of Cannabinoid Designer Drugs, Frontiers in Behavioral Neuroscience, 2011, 5, article number 60.

Huber U., Majors R.E., Principles in Preparative HPLC: A Primer, Agilent Technologies, 2007

Hudson S., Ramsey J., The emergence and analysis of synthetic cannabinoids, Drug Testing and Analysis, 2011, 3(7-8), 466–78.

Jankovics P.,Varadi A., Tolgyesi L., Lohner S., Nemeth-Palotas J., Balla J., Detection and identification of the new potential synthetic cannabinoids 1-pentyl-3-(2-iodobenzoyl)indole and 1-pentyl-3-(1-adamantoyl)indole in seized bulk powders in Hungary, Forensic Science International, 2012, 214(1-3), 27–32.

Logan B.K., Reinhold L. E., Xu A., Diamond F. X., 2012. Identification of synthetic cannabinoids in herbal incense blends in the United States, Journal of Forensic Sciences, 2012, 57(5), 1168–80.

Makriyannis A., Deng H., 2007, Cannabimimetic Indole Derivates, US Patent No. 2080090871A10 2007

Moosmann B., Kneisel S., Girreser U., Brecht V., Separation and structural characterization of the synthetic cannabinoids JWH-412 using GC – MS , NMR analysis and a flash chromatography system, Forensic Science International, 2012, 220, 17–22.

SWGDRUG (2013) SWGDRUG Mass Spectral Library Available online at http://www.swgdrug.org/ms.htm , Accessed on 28.03.2013.

Vardakou I., Pistos C., Spiliopoulou C., Spice drugs as a new trend: mode of action, identification and legislation, Toxicology Letters, 2010, 197(3), 157–62.

Zuba D., Byrska B., Maciow M., Comparison of "herbal highs" composition, Analytical and bioanalytical chemistry, 2011, 400(1), 119–26.