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The emergence and analysis of synthetic cannabinoids

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In late 2008, several synthetic cannabinoids were detected in herbal smoking mixtures. Typical of these products were 'Spice Gold', 'Spice Silver' and 'Yucatan Fire', but many other products have since appeared. The analytes detected, such as JWH-018 and CP47,497 are experimental compounds, some of which were never designed for human use.

Both scientific and anecdotal evidence suggest that these compounds are more potent than traditional cannabis and are being widely used. As a result, authorities around the world are now beginning to control them by either naming individual compounds or using generic legislation.

This, however, is easier said than done as the synthetic cannabinoids detected are constantly changing in attempts by manufacturers to evade legislation.

This paper includes background information in the style of a brief monograph, as an aid to rapidly understanding the pharmacological aspects of these compounds in the forensic context, and then presents a comprehensive set of data, obtained from analysis of purchased products by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Keywords: spice; cannabinoid; AM-694; JWH-018; HU-210; Orbitrap; Cannabicyclohexanol; accurate mass

Introduction

This paper includes background information in the style of a brief monograph, as an aid to rapidly understanding the pharmacological aspects of these compounds in the forensic context, and then presents a comprehensive set of data, obtained from analysis of purchased products by GC-MS and LC-MS/MS.

Background

For centuries *Cannabis sativa* and cannabis extracts have been used in natural medicine. In the 1970s, delta (9)-tetrahydrocannabinol (THC) was found to be the main active ingredient of cannabis, responsible for most of its therapeutic and psychotropic actions. In some countries, cannabis extracts (i.e. sativex) or synthetic drugs based on THC, such as nabilone and dronabinol, are used clinically for the treatment of conditions like chemotherapy-induced nausea and vomiting, multiple sclerosis, and glaucoma.

The side effects of cannabis are well documented[1,2] and the abuse potential of these agents and their synthetic analogues represent a serious limitation in their medical use. In addition, diversion in the use of these active ingredients for recreational purpose is a concern due to their reported enhanced affinities for the cannabinoid receptors.[1,2]

The first evidence of a cannabinoid receptor was suggested by Howlett and Flemming in 1984.[3] In the early 1990s, two pharmacologically distinct cannabinoid receptors (CB_1 CB_2) were cloned and reported.[4,5] The CB_1 receptor is expressed in the peripheral and central nervous system while CB_2 receptors, which play a role in the regulation of the inflammatory process, are located on different types of immune cells and immune-related organs. However, there is evidence that some of the effects of cannabis are not related to either receptor.[6]

Synthetic cannabinoids, more correctly designated as cannabinoid receptor agonists that target the CB_1 receptor, have been investigated and developed over the past 40 years as therapeutic agents, often for the treatment of pain. It has proved difficult, however, to separate the desired properties from unwanted psychoactive effects.

Spice

In late 2008, several synthetic cannabinoids were detected in herbal smoking mixtures often marketed as incense or room odorizers. Typical of these were 'Spice Gold', 'Spice Silver', and 'Yucatan Fire', but many other products later appeared.

These products, of which 'Spice' is perhaps the best known example, have been available globally. Although declared as incense and not for human consumption, Spice-type products are smoked as an apparently legal alternative to cannabis to deliver a so-called 'herbal high'. When analyzed, they have not been found to contain tobacco or cannabis but when smoked, produce effects similar to those of cannabis.

The listed constituents of Spice products often include a long list of plant/herbal ingredients such as Baybean, Blue Lotus, Lion's Tail, Lousewort, Indian Warrior, Dwarf Scullcap, Maconha Brava, Pink Lotus, Marshmallow, Red Clover, Rose, Siberian Motherwort, Vanilla, and Honey. The assumption initially was that it was a botanical ingredient that was responsible

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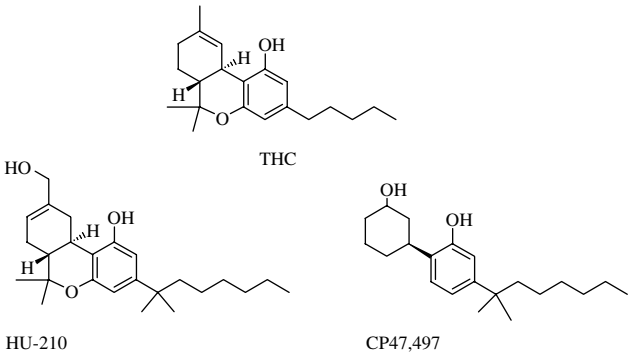


Figure 1. Structures of THC, HU-210-a 'classical cannabinoid' and CP47,497, a 'non-classical cannabinoid'.

for the cannabis-like effect. More recently, however, based on the reported effects of smoking these products, studies have focused on identifying any unlisted components that they may contain.

Synthetic cannabinoids detected in Spice

Several synthetic cannabinoid receptor agonist (cannabinomimetic) compounds have been identified in Spice-type products.

To date these include:

JWH-018[7, 8, 9, personal finding]
JWH-073[10, personal finding]
JWH-250[11, personal finding]
JWH-398[12]
HU-210[13]
CP47,497[8, 9, 14, personal finding]
CP47,497 C8 homologue (cannabicyclohexanol)[8, 9, 14, personal finding]
Cannabicylohexanol + C2 variant[personal finding]
AM694[personal finding]
JWH-122[12, EMCDDA communication, personal communication]
JWH-200[personal finding]
JWH-081[12, EMCDDA communication, personal finding]
JWH-253[personal finding]
JWH-387[personal finding]
JWH-210[EMCDDA communication, personal communication]
JWH-019[personal finding]
JWH-203[EMCDDA communication]
(4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone
personal finding, EMCDDA communication
(4-hydroxymethylphenyl)(1-pentyl-1H-indol-3-yl)methanone
EMCDDA communication

Although often referred to simply as synthetic cannabinoids, many of the substances are not structurally related to the

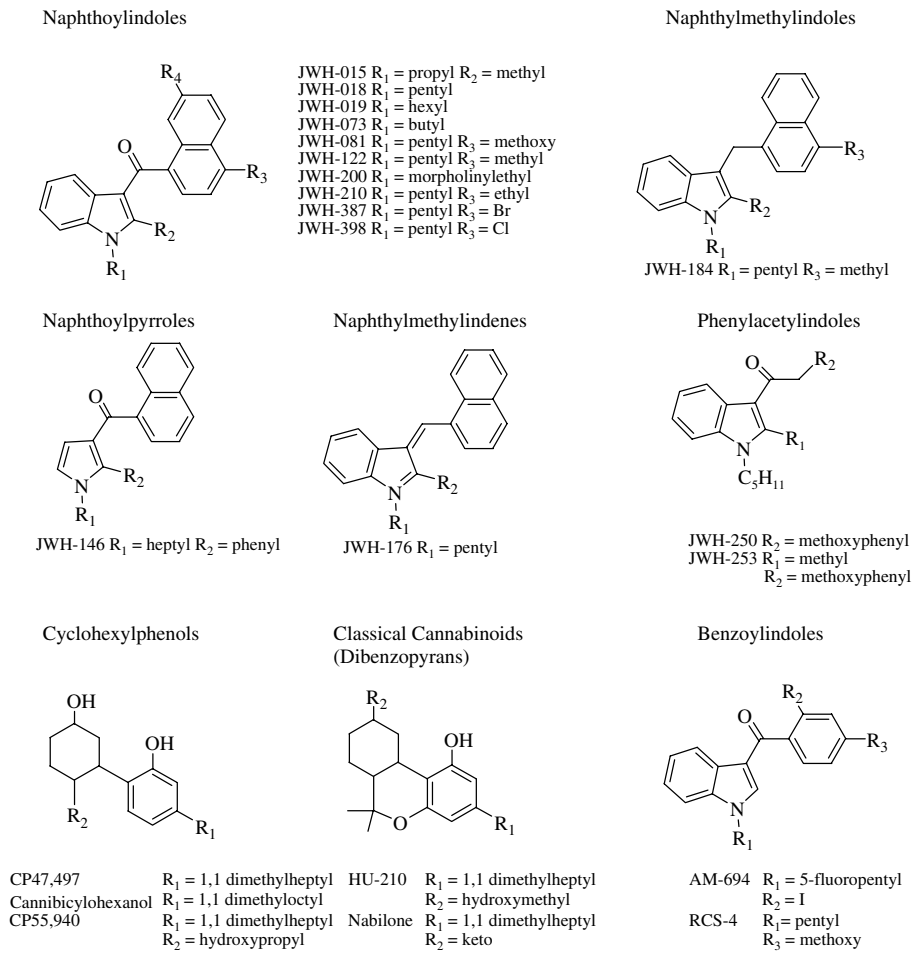
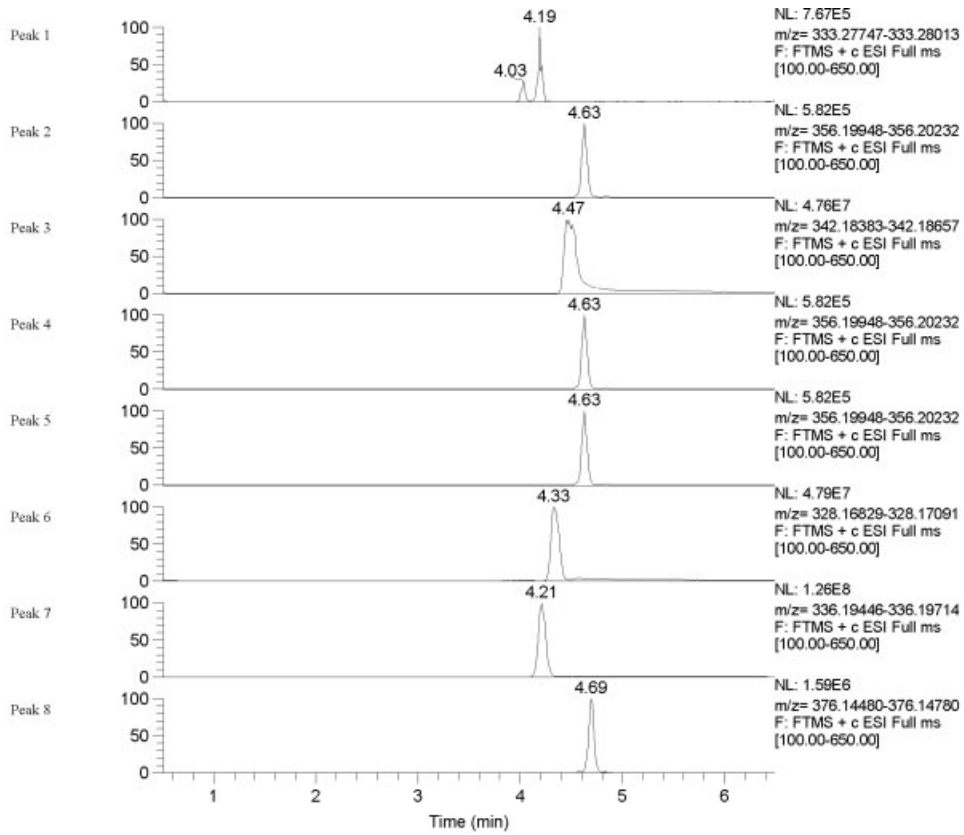


Figure 2. Structures of the main synthetic cannabinoid groups with examples from the data or the ACMD.[13] (R=H unless specified).



Peak Number	Compound Name	Expected m/z	Detected m/z	Delta (mDa)	Delta (ppm)	Expected RT	Actual RT	Intensity
1	CP-47,497-octyl_homolog	333.27880	333.27963	0.8	2.5	4.00	4.19	767189
2	JWH-007	356.20090	356.20157	0.7	1.9	4.00	4.63	581974
3	JWH-018	342.18520	342.18567	0.5	1.4	4.00	4.47	46894419
4	JWH-019	356.20090	356.20157	0.7	1.9	4.00	4.63	581974
5	JWH-047	356.20090	356.20157	0.7	1.9	4.00	4.63	581974
6	JWH-073	328.16960	328.16983	0.2	0.7	4.00	4.33	47726076
7	JWH-250	336.19580	336.19574	-0.1	-0.2	4.00	4.21	125557965
8	JWH-398	376.14630	376.14633	0.0	0.1	4.00	4.69	1589944

Figure 3. High resolution accurate mass screening report for 'Spike 99 Ultra'.

naturally occurring cannabinoids, i.e. compounds, like THC based on dibenzopyran.

Consequently they may be divided into 'classical cannabinoids' structurally similar to THC such as HU-210 and 'non-classical cannabinoids' such as CP47,497, structurally unrelated to THC. Both series of compounds are characterized by a bicyclic structure and are reported in the scientific literature as having been tested successfully for cannabinoid action (Figure 1).

The JWH compounds were developed as test compounds in the investigation of drug-receptor interactions in the study of the endocannabinoid system. The JWH prefix is derived from the initials of John W. Huffman who developed a range of cannabinoid receptor agonists at Clemson University in the USA for researching receptor-drug interactions.

The cannabinoid receptor agonists form a diverse group, but most are lipid soluble and non-polar, and consist of 22 to 26 carbon atoms; they would therefore be expected to volatilize readily when smoked. A common structural feature is a side-chain, where optimal activity requires more than four and up to nine saturated carbon atoms. Most of these compounds can be classified in one of the following groups, although there are a few that fall outside this scheme. The generic structures together with examples from detected compounds are shown in Figure 2.

1. Naphthoylindoles (e.g. JWH-018, JWH-073, JWH-398).
2. Naphthylmethylindoles.
3. Naphthoylpyrroles.
4. Naphthylmethylindenes.
5. Phenylacetylindoles (e.g. JWH-250, JWH-253).
6. Cyclohexylphenols (e.g. CP 47,497, homologues of CP 47,497).
7. Classical cannabinoids (e.g. HU-210, nabilone).
8. Benzoylindoles (e.g AM-694, (4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone

The possible published variants of these compounds are listed in a document from the UK Advisory Council on the Misuse of Drugs (ACMD).[15]

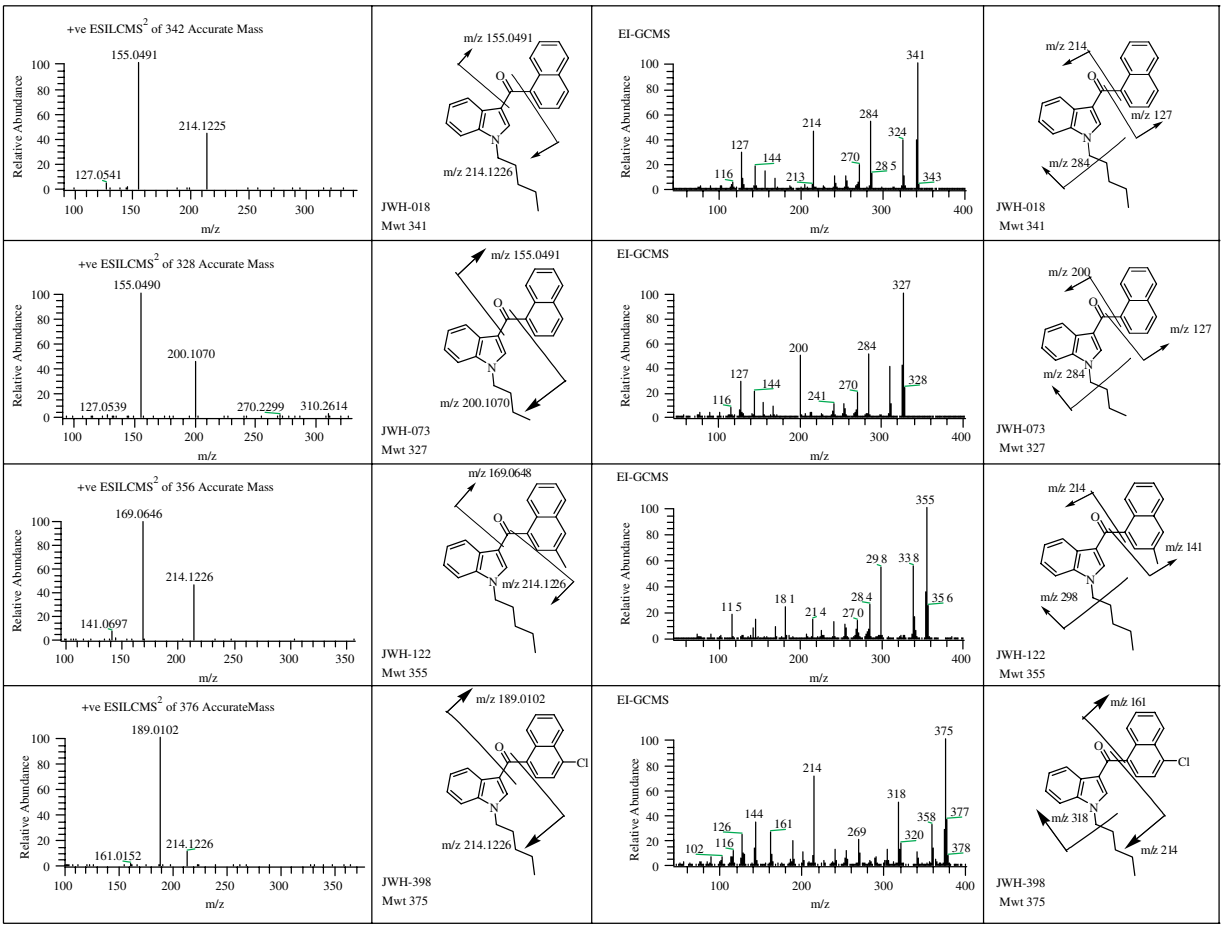


Figure 4. EI GC-MS and accurate mass LC-MS^2 data for JWH-018, JWH-073, JWH-122 and JWH-398.

Some of these compounds have been designated as controlled drugs in countries around the world. It is apparent, however, that as one compound or group of compounds is made illegal, more variants that fall outside current legislation take their place.[10,16]

Effect

There is a considerable volume of anecdotal evidence on various internet sites regarding the efficacy of Spice products with different variants being said to deliver different effects to the user. There is also a documented case where a user reported that he had been smoking Spice Gold daily for eight months. He reported a tolerance to the product which led to increasing the amount he smoked each day. He also felt a conscious desire for the product. On treatment, the physical withdrawal symptoms were very similar to those seen with cannabis dependence.[17] The reported symptoms included profuse sweating leading to internal unrest, a strong desire for Spice, nightmares, nausea, tremor, and headaches. This led to a diagnosis of a dependency syndrome according to both the *International Statistical Classification of Diseases and Related Health Problems 10th revision (ICD-10)* and the *Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV)*.

On admission to hospital, a urine sample was taken. This was subjected to the 'normal' immunological tests performed for cannabinoids, benzodiazepines, amphetamines, cocaine, opiates, and methadone. The results were all negative.

It should be noted that the Spice Gold in this report was not tested for cannabinomimetic compounds or drugs of abuse.

In another study, a self experiment was conducted by two authors.[7] One cigarette containing 0.3g of Spice Diamond was smoked. After approximately 10 min, the first noticeable and cannabis-like effects occurred. These included considerably reddened conjunctivae, a significant increase in pulse rates, xerostomia, and an alteration in mood and perception. In psychomotor tests, no abnormalities were detected, although the subjects had the impression of being moderately impaired. These effects slowly subsided over a period of 6 h. The following day, some minor after effects were reported. Further analytical work on the administered Spice Diamond identified the presence of JWH-018 and the C8 homologue of CP47,497.

A review of clinical reports was recently published in which addiction and withdrawal symptoms similar to those seen with cannabis abuse were linked to the use of Spice.[18]

A huge amount of anecdotal evidence surrounding the effects of Spice and often individual Spice synthetic cannabinoids is available on the internet. For example, the website http://www.bluelight.ru/vb/home.php contains personal reports of use and differences in effects between compounds.

It is apparent that different Spice variants have different active ingredients.[12,14] It is also apparent that the active ingredients

Table 1. Summary of compounds, monoisotopic masses, and data locations

Analyte	Formula	Monoisotopic mass	Source	Figure No.
JWH-018	$C_{24}H_{23}NO$	341.1774	Spice products and customs seizure	**4**
JWH-073	$C_{23}H_{21}NO$	327.1618	Spice products and reference material	**4**
JWH-122	$C_{25}H_{25}NO$	355.1931	'Star of Fire' spice product	**4**
JWH-398	$C_{24}H_{22}ClNO$	375.1384	Numerous spice products	**4**
JWH-015	$C_{23}H_{21}NO$	327.1618	Reference material	**5**
JWH-019	$C_{25}H_{25}NO$	355.1931	Spice product and reference material	**5**
WIN 55212-2	$C_{27}H_{26}N_2O_3$	429.1938	Reference material	**5**
JWH-387	$C_{25}H_{22}BrNO$	419.0879	Spice products	**5**
JWH-200	$C_{25}H_{24}N_2O_2$	384.1832	Customs seizure	**6**
JWH-210	$C_{26}H_{27}NO$	369.2087	Reference material	**6**
JWH-081	$C_{25}H_{25}NO_2$	371.1880	Spice product and reference material	**6**
JWH-250	$C_{22}H_{25}NO_2$	335.1880	Numerous spice products and reference material	**6**
AM-694	$C_{20}H_{19}FINO$	435.0490	Spice products and reference material	**7**
(4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone (RCS-4)	$C_{21}H_{23}NO_2$	321.1723	Jah Rush spice product	**7**
CRA-13	$C_{26}H_{24}O_2$	368.1771	Reference material	**7**
RCS-4 – methylated A	$C_{22}H_{25}NO_2$	335.1880	Jah Rush spice product (minor component)	**7**
RCS-4 – methylated B	$C_{22}H_{25}NO_2$	335.1880	Jah Rush spice product (minor component)	**8**
CP47,497	$C_{21}H_{34}O_2$	318.2553	Numerous spice products and reference material	**8**
Cannabicyclohexanol	$C_{22}H_{36}O_2$	332.2710	Numerous spice products	**8**
Cannabicyclohexanol + C2 variant	$C_{24}H_{40}O_2$	360.2022	Numerous spice products	**8**
CP 55490	$C_{25}H_{40}O_3$	388.2972	Reference material	**9**
HU-308	$C_{27}H_{42}O_3$	414.3128	Reference material	**9**
HU-210	$C_{25}H_{38}O_3$	386.2815	Reference material	**9**
Nabilone	$C_{24}H_{36}O_3$	372.2658	Reference material	**9**
JWH-210 ethyl derivative	$C_{28}H_{31}NO$	397.2400	Minor component of JWH-210 reference material	**10**
JWH-253	$C_{23}H_{27}NO_2$	349.2036	Spice products	**10**
Methylated JWH-073	$C_{24}H_{23}NO$	341.1774	Personal communication	**10**
JWH-200 piperidine variant	$C_{26}H_{26}N_2O$	382.2040	Personal communication	**10**
(4-hydroxymethylphenyl)(1-pentyl-1H-indol-3-yl)methanone	$C_{21}H_{23}NO_2$	321.1723	EMCDDA communication	**10**
JWH-203	$C_{21}H_{22}ClNO$	339.1384	EMCDDA communication	10

may differ in relative amounts from one variant to another and also from batch to batch of the same variant.[12,14]

Legislation

Legislation has been put in place in many countries based on either controlling individual named substances or as in the UK, through the introduction of a generic legislation based on modifications to a core structure as in the recommendation to the UK home office from the ACMD.[15] The position of the World Anti-Doping Agency (WADA) at the time of writing is that synthetic cannabinoids related structurally to traditional cannabinoids only, are prohibited.

Availability

Spice products are readily available on the Internet and in 'head' shops on the high street. Although most now claim to be legal (i.e. free of controlled substances), this is generally not the case, as recent studies in the UK have shown.[16] There have also been several seizures of relatively large amounts (100g or more) of the active substances. It is not clear if these were destined to be added to herbal products for sale or whether they were intended for distribution to the end users as 'white powders'.

Metabolism

Most of the work so far has been performed on the raw ingredient with very little consideration of the detection in mammalian body fluids. A recent publication has shown that both JWH-018 and CP 47,497 can be detected as parent compounds and metabolites after administrations to a rat.[19] Hydroxylation and n-dealkylation occurred with the most abundant analytes being n-dealkylated hydroxylated metabolites. Hydroxylation occurred in both aromatic systems and in the aliphatic side chain. This has more recently also been shown in the human[20,21] and in vitro.[22] The formation of similar metabolites *in vitro* for another naphthoylindole compound JWH-015, had been reported previously.[23]

The metabolism of CP 47,497 in the rat was reported to have been hydroxylation in both aromatic and aliphatic portions of the molecule.

Detection

The synthetic cannabinoids are readily resolved using gas chromatography (GC) and liquid chromatography (LC), but their identification and quantitative analysis is limited by the availability of pure reference materials. Conventional cannabinoid tests utilizing immunoassay are ineffective in detecting these

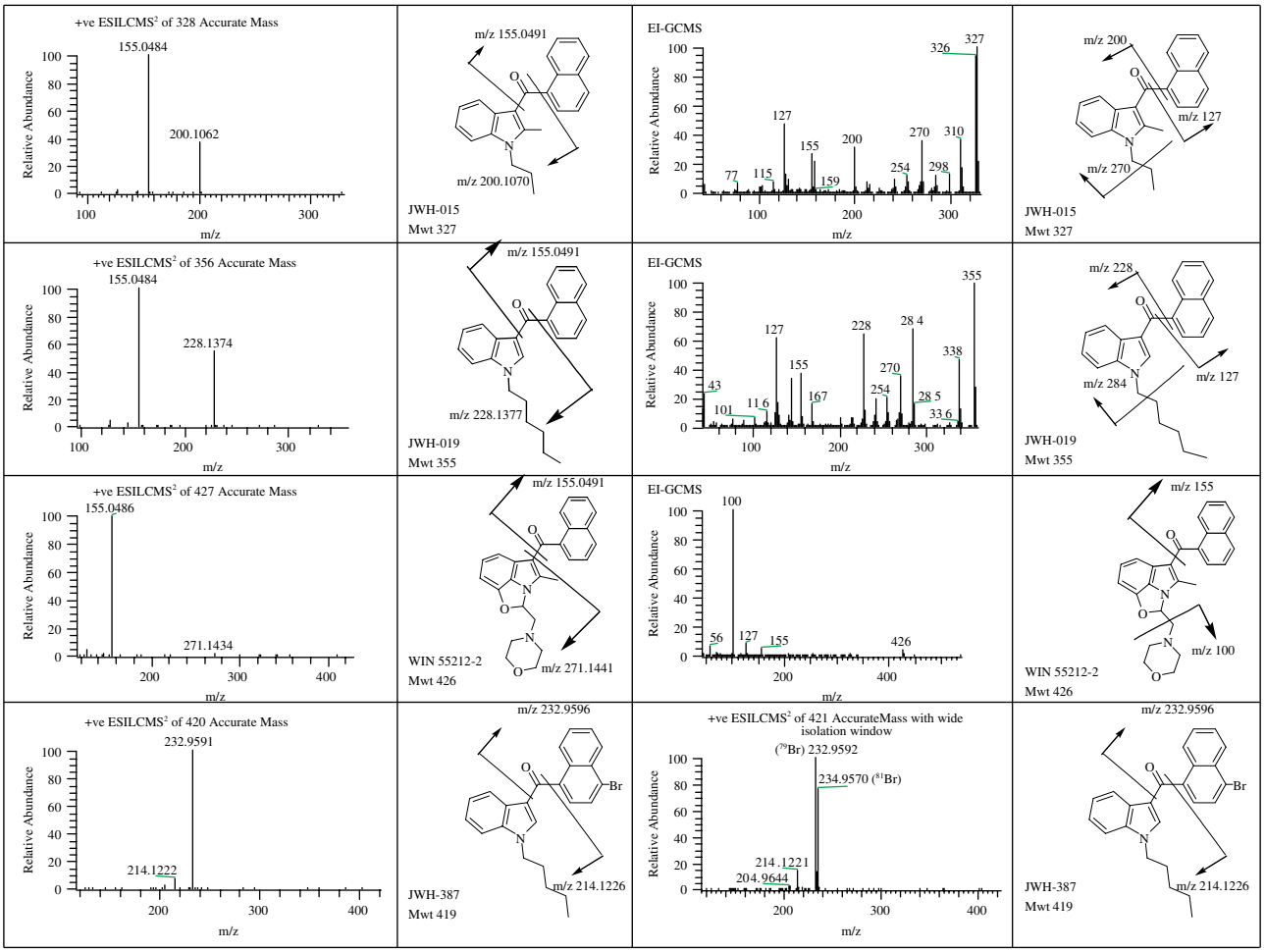


Figure 5. EI GC-MS and accurate mass LC-MS^2 data for JWH-015, JWH-019 and WIN 55212-2. Accurate mass LC-MS^2 for JWH-387.

compounds. Studies on Spice products have generally been performed by GC linked to mass spectrometry (GC-MS)[7,8,9,10,14] or by LC linked to mass spectrometry (LC-MS)[7,8,9,14] but with these technologies, covering the range of over 100 possible compounds without reference materials is difficult. Previous work in our laboratory[12] focused on the use of full-scan high-resolution LC-MS to detect these compounds based on their elemental compositions. Any suspect analytes were then re-analyzed using multistage tandem LC-MS (LC-MS^n) measuring products ions with accurate mass detection. The accurate mass product ions produced helped in the tentative identification of several 'new' compounds.

Monitoring

In a regulatory framework, knowing what is being found in other countries and thereby appreciating which compounds are likely to be seen is very important in maintaining a viable detection service. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) operates an early warning system (EWS) that provides a fast-track mechanism for 30 countries throughout Europe to report new drugs. Any new findings are then communicated back to the relevant organisations in the member countries. In the rapidly evolving world of designer drugs, this resource is invaluable.

We present data from the investigation and analysis of the contents of test-purchased Spice products and seizures by customs and borders agencies. Where available, reference materials have been analyzed and the data included. Additional information was collected and added when not available in-house.

The information presented will act as a reference point for laboratories aiming to detect these compounds in products and body fluids.

Experimental

High performance liquid chromatography (HPLC) grade methanol, toluene, and acetic acid were obtained from ThermoFisher (Loughborough, UK).

N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), ephedrine, and uracil were obtained from Sigma (Poole, UK).

Herbal products were obtained from a variety of sources including police seizures, Internet purchases, and amnesty bins. This was coordinated by TICTAC Communications Ltd (London, UK). Reference materials for JWH-015, JWH-019, WIN55212-2, CP55490, and HU-308 were obtained from Cambridge Bioscience Ltd (Cambridge, UK). HU-210 was obtained from Tocris Bioscience Ltd (Bristol, UK).

Individual compounds were kindly supplied by other workers in this field who are acknowledged accordingly.

Samples of herbal product were prepared for analysis using a simple extraction into methanol. The materials tested did not need any treatment prior to extraction.

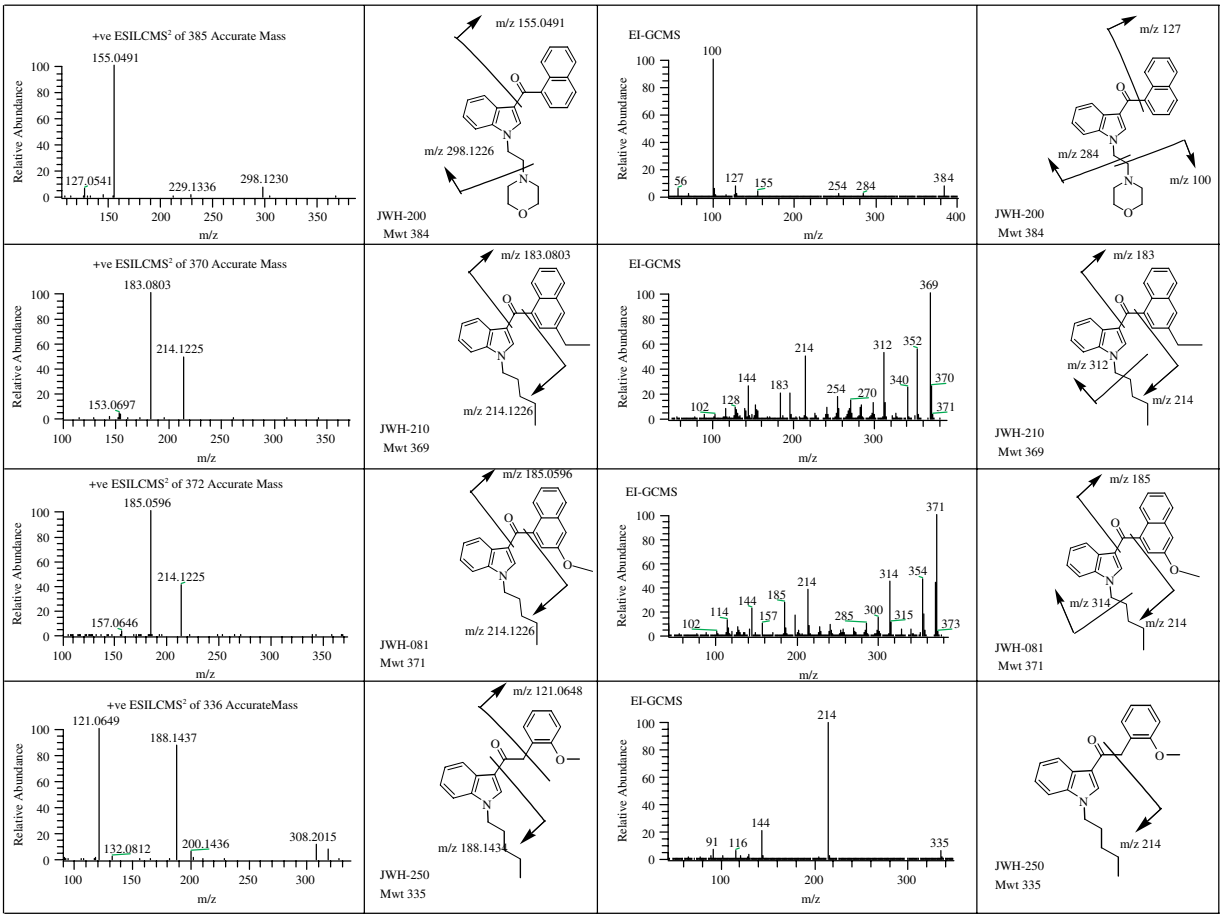


Figure 6. EI GC-MS and accurate mass LC-MS^2 data for JWH-200, JWH-210, JWH-081 and JWH-250.

Approximately 50 mg of the herbal product was placed in a 20-ml glass vial and 2 ml of methanol was added. This was sonicated for 10 min then left for 10 min to settle; 1 ml of the methanol extract was then transferred to a 1-ml glass vial. For all LC-MS analyses, 100 µl of the methanol extract was transferred to a glass autosampler vial followed by the addition of 900 µl of ultrapure water. For GC-MS analysis, 100 µl of the methanol extract was transferred to a GC vial and evaporated to dryness by leaving overnight at room temperature. The sample was reconstituted in 30 µl toluene followed by 20 µl of MSTFA and then submitted for analysis. No heating was performed prior to injection.

Instrumentation

LC-MS

The samples were analyzed on an Accela UPLC system interfaced to an LTQ Orbitrap Discovery both from ThermoFisher (Hemel Hempstead, UK).

The chromatographic separation was performed using a Pursuit Diphenyl 3.0 µm × 2 mm × 100 mm HPLC column from Varian (Oxford, UK) linked to a Waters (Elstree, UK) Acquity in-line filter operating in gradient mode at 40 °C.

The mobile phases were 0.1% acetic acid with 300 µg/l uracil and 0.1% acetic acid in methanol with 300 µg/l uracil. The uracil was added to the mobile phase as a lock mass for the Orbitrap.

The gradient was formed from starting conditions of 80% aqueous mobile phase. Organic mobile phase was increased through the run to give 60% organic at 1.0 min and 99% at 2.5 min. The 99% organic step was held for 3.5 min before reverting to starting conditions at 6.01 min. The flow rate throughout was 400 µl/min.

The LC-MS interface was an Ion Max API source fitted with an electrospray probe. A sheath gas flow rate and auxiliary gas flow rate of 30 and 10 arbitrary units, respectively, were used together with a source voltage of 4.5 KV and a heated capillary transfer temperature of 200 °C. To optimize the remaining source parameters, a 10 µg/ml solution of ephedrine was introduced at a rate of 3 µl/min into a solvent flow of 400 µl/min, consisting of 50% organic mobile phase and 50% aqueous mobile phase. This was achieved using the autotune functionality within the Xcalibur operating software using *m/z* 166 from ephedrine.

The Orbitrap was calibrated for positive ion analysis using the standard ThermoFisher Orbitrap calibration solutions and procedures. The instrument was operated in positive ion mode with a resolution setting of 30 000 with an internal lock mass of 113.03455 derived from uracil in the mobile phase. Data were acquired in full scan mode over a mass range of 50–650 Da.

Further investigation of suspicious peaks

Components identified by accurate mass as possible synthetic cannabinoids based on information from the UK Advisory Council on the Misuse of Drugs[15] and from information from Internet searches were subjected to further investigation by LC-MS^n

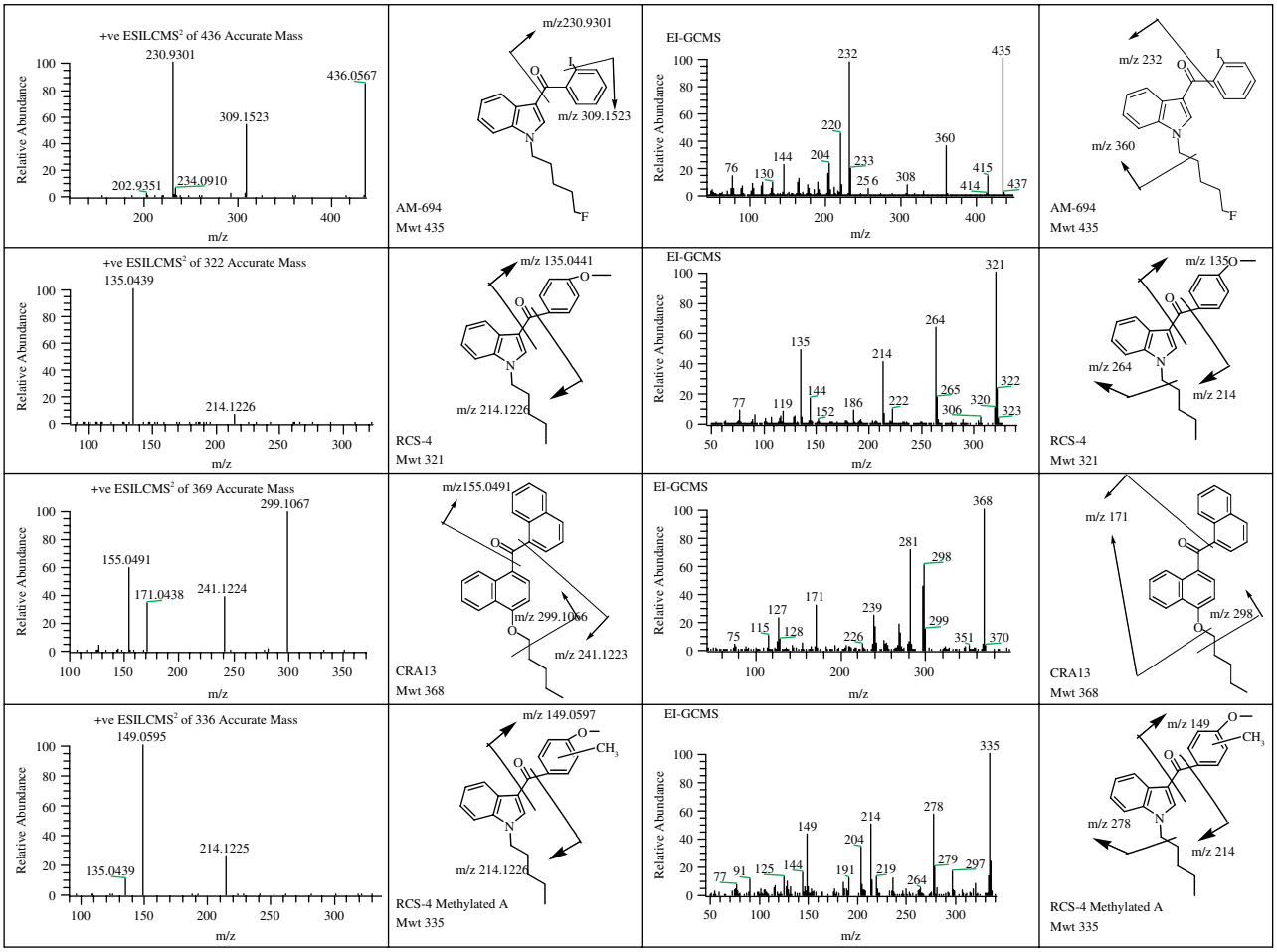


Figure 7. EI GC-MS and accurate mass LC-MS^2 data for AM-694, (4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone (RCS-4), CRA-13 and methylated RCS-4.

analyses in either positive or negative ion mode, depending on which gave the more successful or diagnostic fragmentation. For product ions generated in positive ion mode, the Orbitrap was used to detect the fragments, thereby aiding in structural assignment. Negative ion LC-MS^n was performed using the LTQ using nominal mass measurement. A collision energy of 35 units was used for all positive ion work and 30 units was used for all negative ion work.

GC-MS

The samples were analyzed on an Agilent 6890 GC interfaced to an Agilent 5973 MSD. The chromatographic separation was performed on a 25 metre × 0.25 mm i.d. × 0.25 µm film BPX5 capillary column from SGE. The chromatographic conditions were as follows:

Carrier gas: Helium @15 psi – constant pressure
Injection: Splitless for 1.0 minutes at 280 °C.
Oven: Initial temp 150 °C for 2 min
Ramp 25 °C/min to 230 °C
Ramp 5 °C/min to 330 °C
Hold for 10 min

The mass spectrometer was tuned and calibrated using perfluorotributylamine and data were acquired in full-scan mode over a mass range of 50 to 700Da acquiring spectra at a rate of 4 scans/second. 1 µl of sample was injected on the system

Data processing of full scan accurate mass LC-MS data

Acquired full scan accurate mass LC-MS data were then subjected to analysis using ToxID, a program supplied by ThermoFisher. This utilizes a simple accurate mass database containing compound information including protonated monoisotopic mass.

Narrow range mass filters, typically ±2 ppm, are then employed to selectively filter the data for reporting. In this application, where few retention times are available for the compounds in the database, an expected retention of 4 min was used with a retention window of ±3 min as the instrument run time was 7 min.

The accurate mass database was created for compounds listed in a UK Advisory Council on the Misuse of Drugs communication[15] containing cannabinoid receptor agonists considered for control under the Misuse of Drugs Act 1971 and from other compounds identified on the Internet as being 'synthetic cannabinoids'.

The information in the communication was compiled by reviewing the literature on synthetic cannabinoids over the past 20 years.[24–33] Information on the major substances was extracted. These compounds, in experiments, had all acted as agonists at the CB_1 (cannabinoid) receptor as measured by affinity constants (K_i). Other compounds that have been developed by drug companies

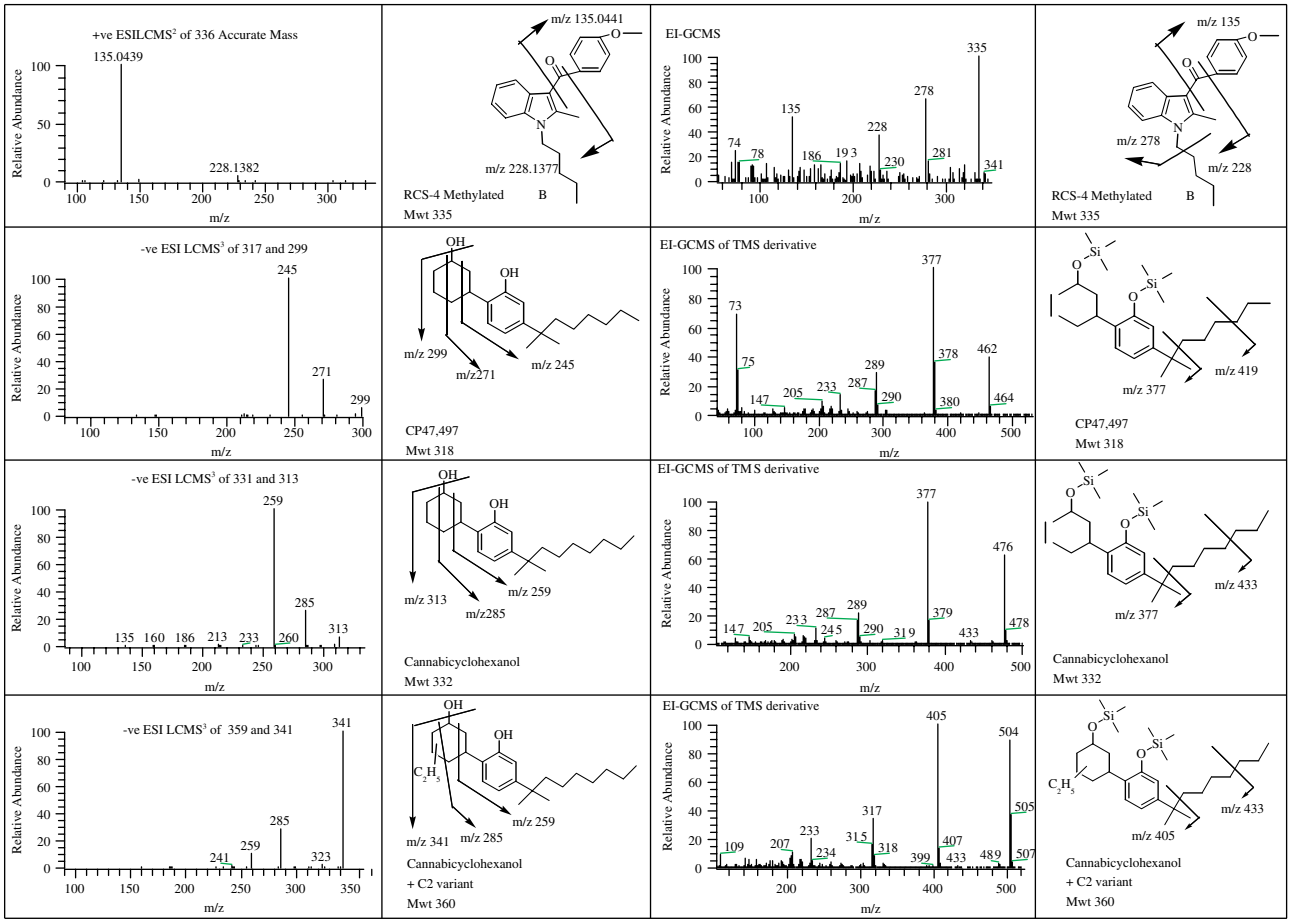


Figure 8. EI GC-MS and accurate mass LC-MS^2 data for methylated RCS-4. EI GC-MS and LC-MS^3 data for CP 47,497, cannabicyclohexanol and cannabicyclohexanol+C2 variant.

as potential synthetic cannabinoids, were also added. A total of 142 compounds were initially included in the database with accurate masses derived from elemental compositions. Not all, however, had high receptor affinities (i.e. low values of K_i); less than half would probably have misuse potential. It may be noted that of the various synthetic cannabinoids already reported, all, like Δ^9-THC itself, have low values of K_i.

Results

The first pass analysis of Spice products was performed using full scan accurate mass LC-MS with data analysis using ToxID and the above database. An example of a report is shown in Figure 3. The data displayed are from an analysis of Spike 99 Ultra and shows the presence of six different spice compounds.

In Figure 3, there is a result for a component at 4.63 min for JWH-007, JWH-019 and JWH-047. These have the same elemental composition and are indistinguishable by accurate mass alone. Follow-up LC-MS^n analyses generating MS^2 and MS^3 data was used to characterize and identify all the analytes. In this case the compound was determined to be JWH-019. Where sufficient analyte existed, full-scan, electronic ionization (EI) GC-MS data were acquired.

The data acquired to date from investigations into Spice products and related compounds are presented in Figures 4, 5, 6, 7, 8, 9 and 10 and are summarized in Table 1.

In addition, a number of reference materials have been analyzed where available, even if not yet detected in Spice products. The data are included as these are not readily available from the literature.

The data includes a reference to cannabicyclohexanol + C2 variant (Figure 8). This was detected in a product called Ice Bud and is the first report of this compound. This was identified with a combination of accurate mass LC-MS, LC-MS^n and full-scan GC-MS of the derivatized and underivatized sample.

All compounds listed are covered by current UK legislation with the exception of AM-694, CRA13, (4-hydroxymethylphenyl)(1-pentyl-1H-indol-3-yl)methanone and (4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone (and its methylated derivatives).

Discussion

It should first of all be noted that most of the compounds reported here have been identified in Spice-type herbal products. These data have been generated through the analysis of acquired materials and in two instances from data circulated by EMCDDA. A few compounds have been acquired as seized powders and purchased reference materials and their analytical data have been included for future reference.

When reviewing accurate mass by only screening data for many of the JWH series of compounds, it is important to remember

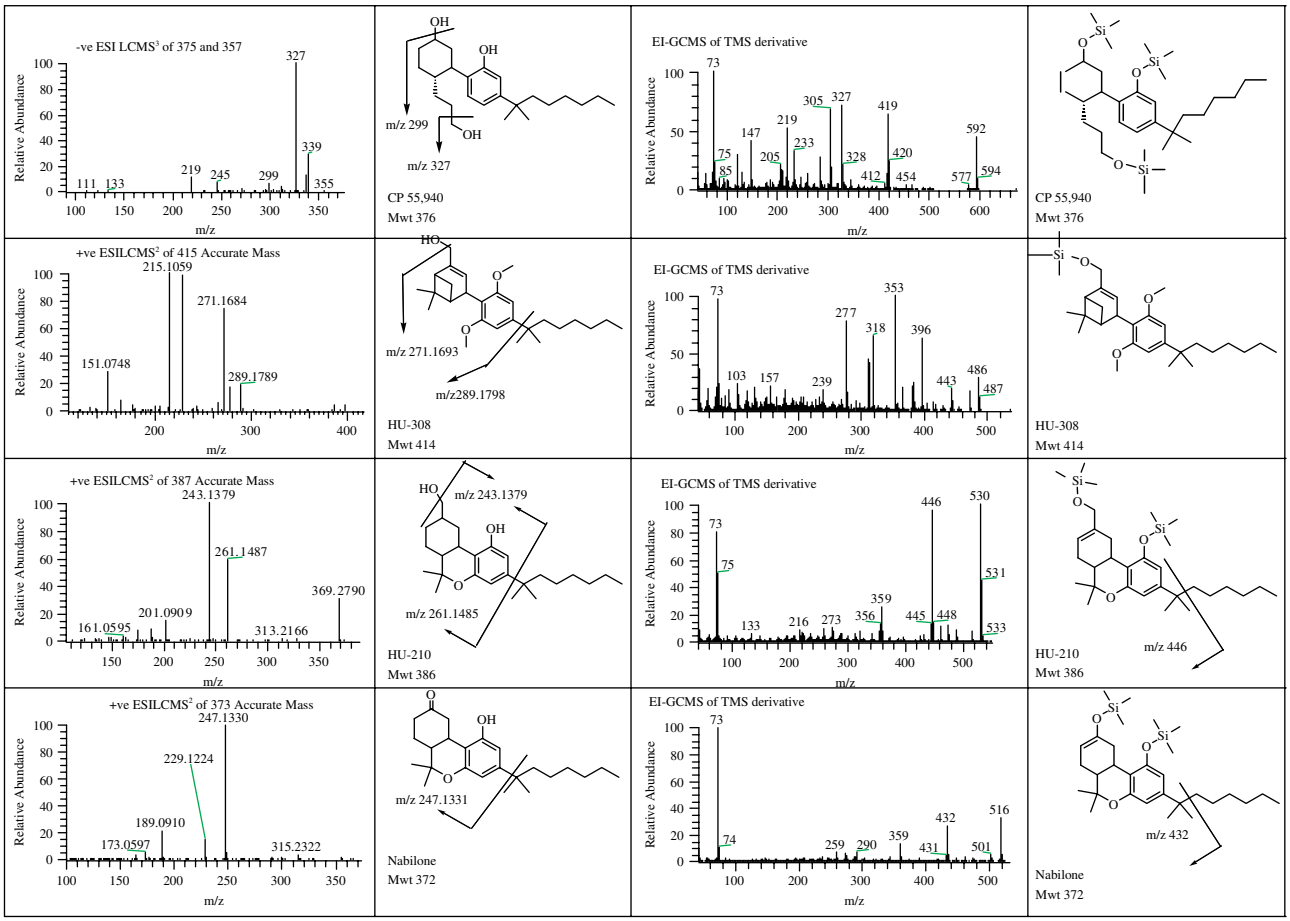


Figure 9. EI GC-MS and LC-MS^3 data for CP55490. EI GC-MS and accurate mass LC-MS^2 data for HU-308, HU-210 and nabilone.

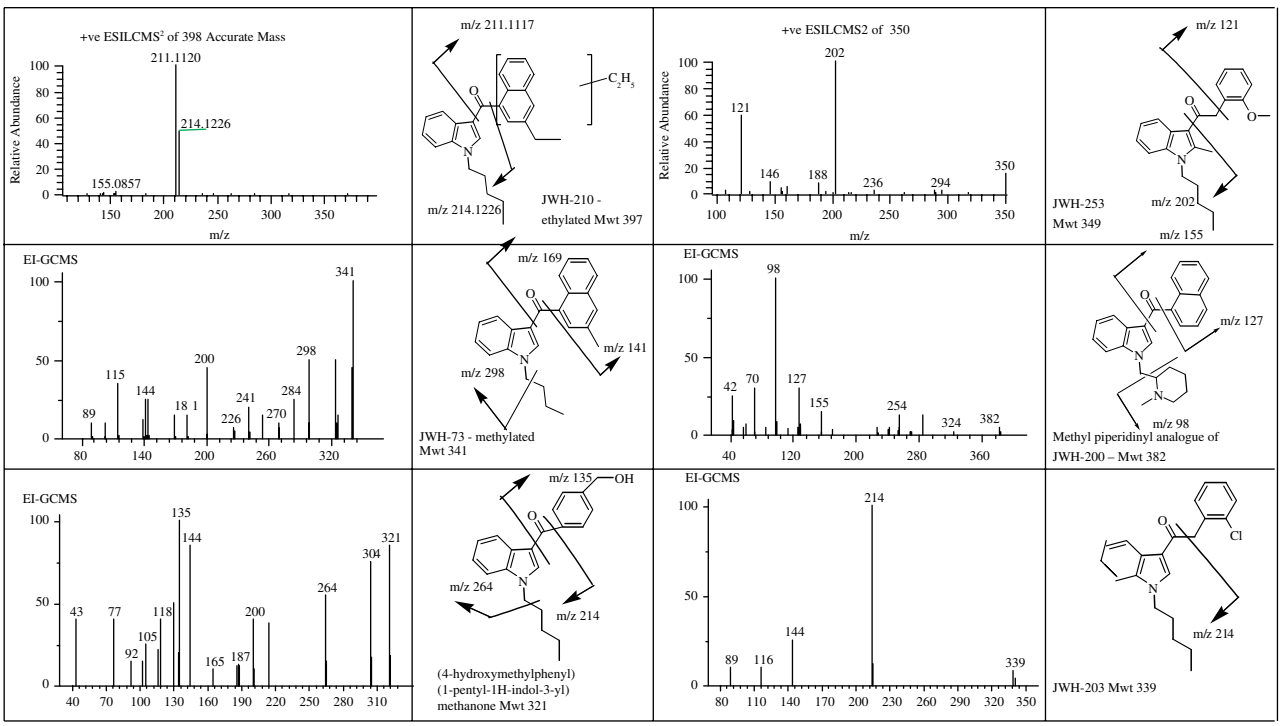


Figure 10. Accurate mass LC-MS^2 data for ethylated JWH-210. LC-MS^2 data for JWH-253. EI GC-MS data for methylated JWH-073, the methylpiperidinyl analogue of JWH-200, (4-hydroxymethylphenyl) (1-pentyl-1H-indol-3-yl) methanone and JWH-203.

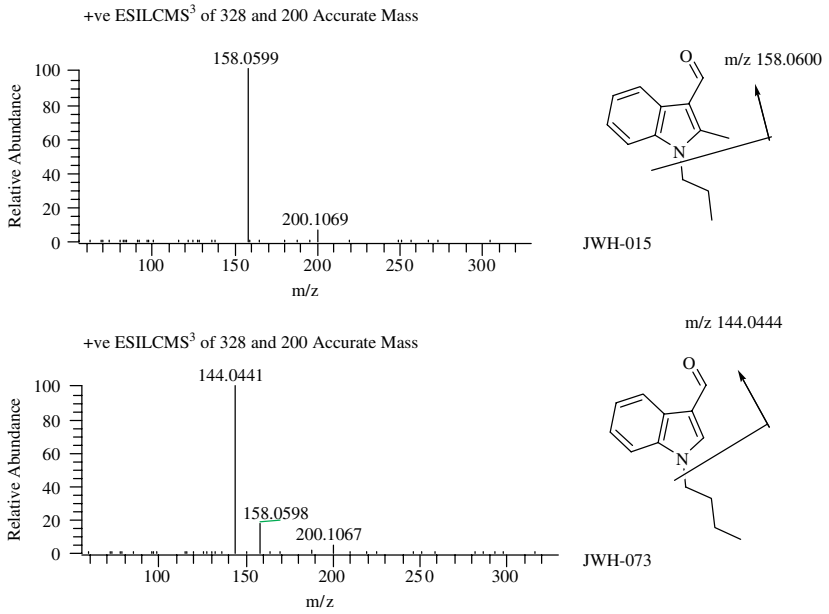


Figure 11. Accurate mass LC-MS^3 data for JWH-015 and JWH-073 showing spectral differences due to positioning of additional methyl group.

that there are several isobars for some elemental compositions. Whereas this is not an issue for a screening analysis as performed here using accurate mass, the differentiation of one isobar from another is very important in a regulatory investigation.

Once a compound has been detected, a follow-up analysis by full-scan EI GC-MS gives more structural information and a greater chance of structural assignment.

Much of the early work in this field, by Auwärter's group[7] and Uchiyama's group[8,9,14] proposed the origin of many of the ions seen in both GC-MS and LC-MS analyses. This information is utilized and built upon in the identification of the synthetic cannabinoids presented here.

As can be seen from both the GC-MS and LC-MS^n data, the naphthoylindole compounds generally fragment in the same manner. The predominant fragment in LC-MS^2 for all these compounds is from the naphthoyl fragment. Any substitution in the naphthoyl portion is seen in a modification to the *m/z* 155 fragment. Exceptions to this include JWH-200 which, due to the morpholinylethyl side chain, fragments differently with predominantly the generation of a morpholinylmethyl fragment of *m/z* 100 in EI GC-MS. The recent report of a piperidinyl version of JWH-200 (Figure 10) follows this pattern with a base peak in the EI GC-MS spectrum of *m/z* 98.

In the case of the isobars JWH-018 and methylated JWH-073, which share the same elemental composition, full-scan high-resolution LC-MS without retention time information would not be able to determine one from the other. EI GC-MS can, however, be used to differentiate these compounds. The *m/z* 284 ion seen in JWH-073 is modified by the addition of the methyl group to the indole portion of the molecule rather than the alkyl side chain, to give an ion of *m/z* 298. The modification of JWH-073, however, to give JWH-018 through the addition of a methyl group to the alkyl side chain would still yield an ion of *m/z* 284.

The use of LC-MS^n can aid in some identifications. In Figure 11, the 'indole' fragments generated by the MS^2 experiment have been subjected to further fragmentation to yield MS^3 spectra. The methyl group in the '2' position adjacent to the alkyl side chain (JWH-015) gives a different MS^3 spectrum to that from an addition of the methyl group to the side chain itself (JWH-073). This knowledge can be used to investigate such structural isomers.

There are some compounds where only one type of data is presented. This is usually where the level is too low for satisfactory analysis by GC-MS. In many of these cases the LC-MS^2 data were acquired on the LTQ rather than the Orbitrap to gain sensitivity. The negative ion LC-MS^3 work on the CP 47,497 compounds was performed on the LTQ element of the LTQ Orbitrap and nominal mass data were obtained.

New compounds are regularly being reported through EMCDDA as new Spice products come onto the market in attempts to evade detection and legislation. A recent example of this was the detection of AM-694 in a seizure of a Spice product from the Glastonbury music festival in the UK. At the time of writing, this is not covered by UK legislation or apparently any European legislation but is a potent and selective agonist for the cannabinoid receptors based on the limited data available which report a high binding affinity (Ki = 0.08 nM) for the CB_1 receptor and an 18-fold selectivity over the CB_2 receptor.[34] Further recent seizures of Spice-type products in the UK contained AM-694 but also contained low levels of JWH-018 and JWH-073. The responses for these two materials were less than 1% of the response of AM-694. When the additional synthetic cannabinoids are at such low levels, they are still readily detectable by both accurate mass LC-MS and LC-MS^2. Detection by GC-MS however is less straightforward as the levels present are not readily detected. This is due in part to the relatively non-volatile nature of the analytes being tested.

Recently, a new naphthoylindole, similar to JWH-200 but with a piperidine ring rather than the morpholine ring was identified (pers. comm.). This compound is not covered by current UK legislation and does not feature in the literature.

Many of the samples submitted for analysis in the authors' laboratory appeared to contain small amounts of the naturally occurring cannabinoid agonist oleamide. This may originate in the plant material that forms the bulk of the Spice product. One sample, however, had a level of oleamide that was between 1000

and 10 000 times greater than that seen as probable background. Crudely, based on the response for 10 mg/gram for JWH-018, the level was in excess of 100 mg/gram of product. Whether or not this product had been fortified with oleamide is a point of debate

From an anti-doping in sport and workplace drug testing perspective, details around the metabolism of these compounds in the human are beginning to emerge. The recent paper on JWH-018[20] details how this compound is metabolized and further work *in vitro* has supported this.[22] This information can be used to determine the possible metabolites of many of the related compounds presented here. The metabolism of CP47,497 has also been reported and once again this information gives a starting point for possible detection by laboratories. Very recently, there was a report of findings of JWH-018 metabolites in doping control samples.[21]

These drugs are not currently covered by the WADA 2010 prohibited list[35] except for those based on the traditional cannabinoid structure, such as nabilone and HU-210. The new prohibited list for 2011 does include other compounds, specifically JWH-018 and JWH-073.[35] The generic term 'cannabimimetic' could be used effectively to cover variants without having to put in place complex legislation such as the December 2009 modification to the Misuse of Drugs Act in the UK.

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