Recommended methods for the **Identification and Analysis of Synthetic Cannabinoid Receptor Agonists in Seized Materials**

MANUAL FOR USE BY NATIONAL DRUG ANALYSIS LABORATORIES

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Note

Operating and experimental conditions are reproduced from the original reference materials, including unpublished methods, validated and used in selected national laboratories as per the list of references. A number of alternative conditions and substitution of named commercial products may provide comparable results in many cases, but any modification has to be validated before it is integrated into laboratory routines.

Mention of names of firms and commercial products does not imply the endorsement of the United Nations.

ST/NAR/48

Acknowledgements

Contents

1. Introduction

1.1 Background

In 2008, several synthetic cannabinoid receptor agonists (referred to as "synthetic cannabinoids" throughout the rest of this document) were detected in herbal smoking blends which were sold on the Internet and in specialized shops under a variety of brand names such as "Spice Silver", "Spice Gold", "Spice Diamond", "Yucatan Fire" and "Smoke" [1, 2]. These colourful and professionally designed packages of herbal products typically contain about 0.5-3 grams of finely cut plant material to which one or more synthetic cannabinoids have been added [3,4]. Generally, they do not contain cannabis but produce cannabis-like effects. Furthermore, they are usually administered by smoking, either as a joint or in a water pipe.

Before 2008, the use of these herbal products seemed to be restricted to a small number of experimental users. However, in 2008, these products achieved immense popularity in Germany and other European countries through the Internet and in subsequent media reports, where they were referred to as "legal alternatives" to cannabis, thus unintentionally promoting the use of these drugs. Since then, hundreds of new herbal products with different brand names have been marketed. The synthetic additives in these products could vary significantly in terms of quantity as well as the types of synthetic cannabinoids used [2, 3, 5-19].

Although so far, relatively little is known about the pharmacology and toxicology of the various (frequently changing) synthetic cannabinoids that are added to the herbal products, a number of these substances may have a higher addictive potential compared to cannabis due to quicker development of tolerance and could exhibit a tendency towards higher acute and long-term toxicity.

Currently, none of the synthetic cannabinoids found in these herbal products are internationally controlled by the 1961 Single Convention on Narcotic Drugs or by the 1971 Convention on Psychotropic Substances. Moreover, the control status of these compounds differs significantly from country to country. Most countries are challenged by the sheer number of synthetic cannabinoids constantly emerging, which means that control measures targeting individual compounds can be easily

circumnavigated. At the time of publication, some Member States, for example, Austria, Ireland, Luxembourg, Switzerland and United Kingdom, have adopted a more generic approach to controlling synthetic cannabinoids of similar structures. Nevertheless, effective implementation of control measures could be hampered by the lack of analytical data and reference standards.

1.2   Purpose and use of the *Manual*

The present *Manual* is one in a series of similar publications dealing with the identification and analysis of various types of drugs under control. These manuals are the outcome of a programme pursued by UNODC since the early 1980s, aimed at the harmonization and establishment of recommended methods of analysis for national drug analysis laboratories.

In line with the overall objective of the series, the present *Manual* suggests approaches that may assist drug analysts in the selection of methods appropriate to the sample under examination and provide data suitable for the purpose at hand, leaving room also for adaptation to the level of sophistication of different laboratories and the various legal needs. The majority of methods included in the present *Manual* are validated methods, which have been used in reputable laboratories. The reader should be aware, however, that there are a number of other methods, including those published in the forensic science literature, which may also produce acceptable results. **Any new method that is about to be used in the reader's laboratory must be validated and/or verified prior to routine use.**

In addition, there are a number of more sophisticated approaches, but they may not be necessary for routine operational applications. Therefore, the methods described here should be understood as guidance, that is, minor modifications to suit local circumstances should not normally change the validity of the results. The choice of the methodology and approach to analysis as well as the decision whether or not additional methods are required remain with the analyst and may also be dependent on the availability of appropriate instrumentation and the level of legally acceptable proof in the jurisdiction within which the analyst works.

Attention is also drawn to the vital importance of the availability to drug analysts of reference materials and literature on drugs of abuse and analytical techniques. Moreover, the analyst must of necessity keep abreast of current trends in drug analysis, consistently following current analytical and forensic science literature.

UNODC's Laboratory and Scientific Section would welcome observations on the contents and usefulness of the present *Manual*. Comments and suggestions may be addressed to:

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All manuals, as well as guidelines and other scientific-technical publications may be requested by contacting the address above.

2. General aspects

2.1 Definition of synthetic cannabinoids

Synthetic cannabinoids are referred to as substances with structural features which allow binding to one of the known cannabinoid receptors, i.e. CB_1 or CB_2, present in human cells. The CB_1 receptor is located mainly in the brain and spinal cord and is responsible for the typical physiological and particularly the psychotropic effects of cannabis, whereas the CB_2 receptor is located mainly in the spleen and cells of the immune system and may mediate immune-modulatory effects.

With the exception of endocannabinoids, naturally occurring cannabinoids are limited to chemical constituents of cannabis such as Δ^9-tetrahydrocannabinol and cannabidiol. In contrast, synthetic cannabinoids as defined above, could encompass a great variety of structurally dissimilar compounds with the possibility for further structural changes, i.e. analogues and derivatives, which could potentially show affinity to either one of the cannabinoid receptors as well.

The binding of synthetic cannabinoids to cannabinoid receptors may result in (partial) agonistic, inverse agonistic or antagonistic effects. Synthetic cannabinoids of interest in forensic science contexts are mainly compounds showing sufficient affinity to the CB_1 receptor and show agonistic or partial agonistic activity as the typical psychotropic cannabis-like effects are mediated typically via agonistic stimulation of this receptor type.

2.2 Chemical classification

Cannabinoid receptor agonists can be classified based on their chemical structures into the following main groups [20]:

1. *Classical cannabinoids*
    Tetrahydrocannabinol, other chemical constituents of cannabis and their structurally related synthetic analogues, e.g. AM-411, AM-906, HU-210, O-1184

2.   *Non-classical cannabinoids*
     Cyclohexylphenols or 3-arylcyclohexanols, e.g. CP-55,244, CP-55,940, CP-47,497
     (and C6-9 homologues)

3.   *Hybrid cannabinoids*
     Combinations of structural features of classical and non-classical cannabinoids,
     e.g. AM-4030

4.   *Aminoalkylindoles*, which can be further divided into the following groups:

     (a)   Naphthoylindoles (e.g. JWH-015, JWH-018, JWH-073, JWH-081, JWH-122,
             JWH-200, JWH-210, JWH-398)

     (b)   Phenylacetylindoles (e.g. JWH-250, JWH-251)

     (c)   Benzoylindoles (e.g. pravadoline, AM-694, RSC-4)

     (d)   Naphthylmethylindoles (e.g. JWH-184)

     (e)   Cyclopropoylindoles (e.g. UR-144, XLR-11)

     (f)   Adamantoylindoles (e.g. AB-001, AM-1248)

     (g)   Indole carboxamides (e.g. APICA, STS-135)

5.   *Eicosanoids*
     Endocannabinoids such as anandamide (AEA), and their synthetic analogues,
     e.g. methanandamide (AM-356)

6.   *Others*
     Encompassing other structural types such as diarylpyrazoles (e.g. Rimonabant®),
     naphthoylpyrroles (e.g. JWH-307, [21, 22]), naphthylmethylindenes (e.g. JWH-176)
     and indazole carboxamides (e.g. APINACA, [23]).

Many derivatives and analogues in the above classes of compounds could be syn-
thesized by the addition of a halogen, alkyl, alkoxy or other substituents to one of
the aromatic ring systems. Other small changes such as variation of the length and
configuration of the alkyl chain can also be made. The aminoalkylindoles are by
far the most prevalent class of synthetic cannabinoids found in herbal products as
they are easier to synthesize, compared to the other classes of compounds.

2.3   Products and modes of administration

A few synthetic cannabinoids such as CP-55,940 or WIN-55,212-2 were commer-
cially available as research chemicals in small quantities many years before the
appearance of such compounds in "ready-to-smoke" products. They were almost
exclusively used in pharmacological research.

Around 2004, the first products containing synthetic cannabinoids emerged. They were added to plant material, e.g. crushed leaves or strips of leaves, by soaking or spraying a solution of one or more synthetic cannabinoids in an organic solvent which was later evaporated. In some cases, synthetic cannabinoids in solid form (crystalline powder) were used, leading to an inhomogeneous distribution of the active compound in the plant material. A minority of these products was found to resemble hashish in colour and texture. They are used similarly to hashish, i.e. mixed with tobacco in a joint or smoked pure in a pipe.

In recent years, a growing number of online shops and traders have started to offer synthetic cannabinoids as "research chemicals" in variable amounts from milligram to kilogram quantities. These substances are not only procured by mass producers of these herbal products but also by end users who would concoct their own blend of herbal mixtures. Some of these substances were of high purity [24], while others were contaminated with synthetic by-products or artefacts due to insufficient clean-up [18].
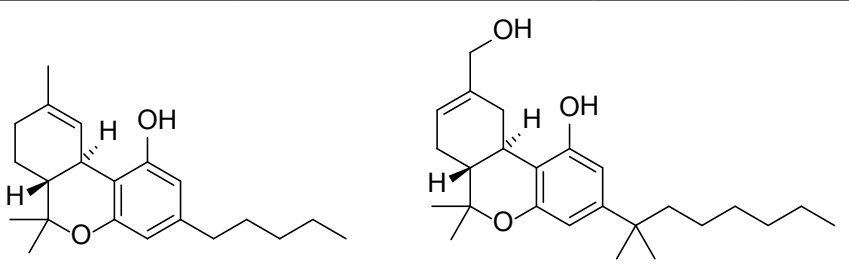
Aside from smoking, there are few reports on the oral consumption of these herbal products containing synthetic cannabinoids taken with food or prepared as a tea. Other means of administration such as intravenous injection or snorting have not been reported to play a significant role.

3. Description of the pure compounds
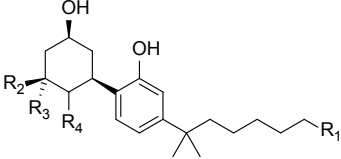
The pure compounds are mostly in the form of fine crystalline powders with colours ranging from white to a grey, brownish or yellowish hue. Most of the compounds are highly lipophilic and show good solubility in solvents with low polarity (e.g. isooctane) as well as in methanol, ethanol, acetonitrile, ethyl acetate, acetone and other medium polar organic solvents. Generally, water solubility of the synthetic cannabinoids used in herbal products is low.

The following list encompasses active compounds which were found either in herbal products or were seized as bulk powders in the respective classes as defined in section 2.2.

3.1 Classical cannabinoids

THC   HU-210

Name	Chemical name	CAS No	Molecular formula
THC *Synonym:* Δ9-Tetrahydrocannabinol	(6a*R*,10a*R*)-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[b,d]pyran-1-ol	1972-08-3	$C_{21}H_{30}O_2$
HU-210 *Synonym:* 11-Hydroxy-Δ8-THC-DMH	(6a*R*,10a*R*)-6a,7,10,10a-tetrahydro-6,6-dimethyl-9-(hydroxymethyl)-3-(2-methyloctan-2-yl)-6*H*-dibenzo[b,d]pyran-1-ol	112830-95-2	$C_{25}H_{38}O_3$

3.2   Non-classical cannabinoids

CP-47,497 ($R_2=R_3=R_4=H$, $R_1=$methyl)

CP-47,497-C6 ($R_1=R_2=R_3=R_4=H$)

CP-47,497-C8 ($R_2=R_3=R_4=H$, $R_1=$ethyl)

CP-47,497-C9 ($R_2=R_3=R_4=H$, $R_1=$propyl)

CP-55,940 ($R_2=R_3=H$, $R_1=CH_3$, $R_4=$3-hydroxypropyl)

Dimethyl CP-47,497-C8 ($R_2=R_3=CH_3$, $R_4=H$, $R_1=$ethyl)

Name	Chemical name	CAS No	Molecular formula
CP-47,497	*rel*-2[(1*S*,3*R*)-3-hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol	70434-82-1	$C_{21}H_{34}O_2$
CP-47,497-C6	*rel*-2[(1*S*,3*R*)-3-hydroxycyclohexyl]-5-(2-methylheptan-2-yl)phenol	not available	$C_{20}H_{32}O_2$
CP-47,497-C8 *Synonym:* Cannabicyclohexanol	*rel*-2-[(1*S*,3*R*)-3-hydroxycyclohexyl]-5-(2-methylnonan-2-yl)phenol	70434-92-3	$C_{22}H_{36}O_2$
CP-47,497-C9	*rel*-2[(1*S*,3*R*)-3-hydroxycyclohexyl]-5-(2-methyldecan-2-yl)phenol	not available	$C_{23}H_{38}O_2$
CP-55,940	*rel*-2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol	83003-12-7	$C_{24}H_{40}O_3$
Dimethyl CP-47,497-C8	*rel*-2-[(1*S*,3*R*)-3-hydroxy-5,5-dimethylcyclohexyl]-5-(2-methylno-nan-2-yl)phenol	not available	$C_{24}H_{40}O_2$

3.3   Hybrid cannabinoids

No compounds have been seized yet in this category.

3.4 Aminoalkylindoles

(a) Naphthoylindoles

$R_1=R_3=H$

AM-1220 (R_2=1-methylpiperidin-2-yl)

AM-2201 (R_2=4-fluorobutyl)

AM-2232 (R_2=butanenitrile)

JWH-018 (R_2=butyl)

JWH-019 (R_2=pentyl)

JWH-020 (R_2=hexyl)

JWH-022 (R_2=3-buten-1-yl)

JWH-072 (R_2=ethyl)

JWH-073 (R_2=propyl)

JWH-200 (R_2=4-morpholinylmethyl)
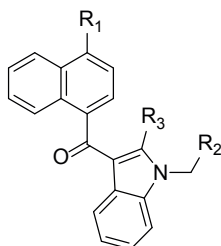
R_2=butyl, R_3=H

JWH-081 (R_1=methoxy)

JWH-122 (R_1=methyl)

JWH-210 (R_1=ethyl)

JWH-387 (R_1=Br)

JWH-398 (R_1=Cl)

JWH-412 (R_1=F)

JWH-007 (R_1=H, R_2=butyl, R_3=methyl)

JWH-015 (R_1=H, R_2=ethyl, R_3=methyl)

JWH-073 4-methylnaphthyl (R_1=methyl, R_2=propyl, R_3=H)

MAM-2201 (R_1=methyl, R_2=4-fluorobutyl, R_3=H)

Name	Chemical name	CAS No	Molecular formula
AM-1220	(naphthalen-1-yl)[1-[(1-methylpiperidin-2-yl)methyl]-1*H*-indol-3-yl]methanone	137642-54-7	$C_{26}H_{26}N_2O$
AM-1220 azepane isomer	(naphthalen-1-yl)[1-(1-methylazepan-3-yl)-1*H*-indol-3-yl]methanone	not available	$C_{26}H_{26}N_2O$
AM-2201	(naphthalen-1-yl)[1-(5-fluoropentyl)-1*H*-indol-3-yl]methanone	335161-24-5	$C_{24}H_{22}FNO$
AM-2232	5-(3-(1-naphthoyl)-1*H*-indol-1-yl)pentanenitrile	335161-19-8	$C_{24}H_{20}N_2O$
JWH-007	(naphthalen-1-yl)(2-methyl-1-pentyl-1*H*-indol-3-yl)methanone	155471-10-6	$C_{25}H_{25}NO$
JWH-015	(naphthalen-1-yl)(2-methyl-1-propyl-1*H*-indol-3-yl)methanone	155471-08-2	$C_{23}H_{21}NO$
JWH-018 *Synonym:* AM678	(naphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone	209414-07-3	$C_{24}H_{23}NO$
JWH-019	(naphthalen-1-yl)(1-hexyl-1*H*-indol-3-yl)methanone	209414-08-4	$C_{25}H_{25}NO$
JWH-020	(naphthalen-1-yl)(1-heptyl-1*H*-indol-3-yl)methanone	209414-09-5	$C_{26}H_{27}NO$
JWH-022	(naphthalen-1-yl)[1-(pent-4-en-1-yl)-1*H*-indol-3-yl]methanone	209414-16-4	$C_{24}H_{21}NO$

JWH-072	(naphthalen-1-yl)(1-propyl-1*H*-indol-3-yl)methanone	209414-06-2	$C_{22}H_{19}NO$
JWH-073	(naphthalen-1-yl)(1-butyl-1*H*-indol-3-yl)methanone	208987-48-8	$C_{23}H_{21}NO$
JWH-073 (4-methylnaph-thyl) *Synonym:* JWH 122 N-butyl analogue	(4-methylnaphthalen-1-yl)(1-butyl-1*H*-indol-3-yl)methanone	1354631-21-2	$C_{24}H_{23}NO$
JWH-081	(4-methoxynaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone	210179-46-7	$C_{25}H_{25}NO_2$
JWH-122 [5]	(4-methylnaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone	619294-47-2	$C_{25}H_{25}NO$
JWH-200 *Synonym:* WIN 55,225	(naphthalen-1-yl)[1-[2-(morpholin-4-yl)ethyl]-1*H*-indol-3-yl]methanone	103610-04-4	$C_{25}H_{24}N_2O_2$
JWH-210	(4-ethylnaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone	824959-81-1	$C_{26}H_{27}NO$
JWH-387	(4-bromonaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone	207227-49-4	$C_{24}H_{22}BrNO$
JWH-398	(4-chloronaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone	1292765-18-4	$C_{24}H_{22}ClNO$
JWH-412	(4-fluoronaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)methanone	1364933-59-4	$C_{24}H_{22}FNO$
MAM-2201 *Synonyms:* JWH-122 (5-fluoropentyl); AM-2201 4-methylnaphthyl analogue	(4-methylnaphthalen-1-yl)[1-(5-fluoro-pentyl)-1*H*-indol-3-yl]methanone	1354631-24-5	$C_{25}H_{24}FNO$

(b) Phenylacetylindoles
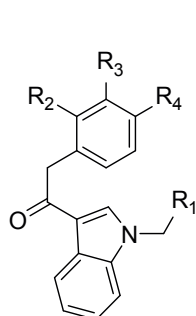
R$_3$=R$_4$=H

Cannabipiperidiethanone (R$_1$=1-methylpiperidin-2-yl, R$_2$=methoxy)

JWH-203 (R$_1$=butyl, R$_2$=Cl)

JWH-250 (R$_1$=butyl, R$_2$=methoxy)

JWH-251 (R$_1$=butyl, R$_2$=methyl)

RCS-8 (R$_1$=cyclohexylmethyl, R$_2$=methoxy)

R$_1$=butyl, R$_2$=H

JWH-201 (R$_3$=H, R$_4$=methoxy)

JWH-302 (R$_3$=methoxy, R$_4$=H)

Name	Chemical name	CAS No	Molecular formula
Cannabipiperidiethanone *Synonym:* JWH-250 1-(2-methylene-*N*-methyl-piperidyl) derivative	2-(2-methoxyphenyl)-1-[1-[(1-methylpiperidin-2-yl)methyl]-1*H*-indol-3-yl]ethanone	1345970-43-5	C$_{24}$H$_{28}$N$_2$O$_2$
JWH-201 *Synonym:* para-JWH-250	2-(4-methoxyphenyl)-1-(1-pentyl-1*H*-indol-3-yl)ethanone	864445-47-6	C$_{22}$H$_{25}$NO$_2$
JWH-203	2-(2-chlorophenyl)-1-(1-pentyl-1*H*-indol-3-yl)ethanone	864445-54-5	C$_{21}$H$_{22}$ClNO
JWH-250	2-(2-methoxyphenyl)-1-(1-pentyl-1*H*-indol-3-yl)ethanone	864445-43-2	C$_{22}$H$_{25}$NO$_2$
JWH-251	2-(2-methylphenyl)-1-(1-pentyl-1*H*-indol-3-yl)ethanone	864445-39-6	C$_{22}$H$_{25}$NO
JWH-302 *Synonym:* meta-JWH-250	2-(3-methoxyphenyl)-1-(1-pentyl-1*H*-indol-3-yl)ethanone	864445-45-4	C$_{22}$H$_{25}$NO$_2$
RCS-8 *Synonyms:* SR-18; BTM-8	2-(2-methoxyphenyl)-1-(1-(2-cyclohexylethyl)-1*H*-indol-3-yl)ethanone	1345970-42-4	C$_{25}$H$_{29}$NO$_2$

(c) Benzoylindoles
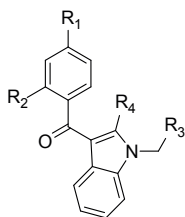
AM-694 (R$_1$=R$_4$=H, R$_2$=I, R$_3$=4-fluorobutyl)

AM-694 chloro derivative (R$_1$=R$_4$=H, R$_2$=I, R$_3$=4-chlorobutyl)

AM-2233 (R$_1$=R$_4$=H, R$_2$=I, R$_3$=1-methylpiperidin-2-yl)

RCS-4 (R$_1$=methoxy, R$_2$=R$_4$=H, R$_3$=butyl)

RCS-4-ortho isomer (R$_1$=R$_4$=H, R$_2$=methoxy, R$_3$=butyl)

RCS-4 butyl homolog (R$_1$=methoxy, R$_2$=R$_4$=H, R$_3$=propyl)

WIN 48,098 (R$_1$=methoxy, R$_2$=H, R$_3$=4-morpholinylmethyl, R$_4$=methyl)

Name	Chemical name	CAS No	Molecular formula
AM-694	(2-iodophenyl)[1-(5-fluoropentyl)-1*H*-indol-3-yl]methanone	335161-03-0	C$_{20}$H$_{19}$FINO
AM-694 (chloro derivative)	(2-iodophenyl)[1-(5-chloropentyl)-1*H*-indol-3-yl]methanone	not available	C$_{20}$H$_{19}$ClINO
AM-2233	(2-iodophenyl)[1-[(1-methylpiperidin-2-yl)methyl]-1*H*-indol-3-yl]methanone	444912-75-8	C$_{22}$H$_{23}$IN$_2$O
RCS-4 Synonyms: SR-19; OBT-199; BTM-4; E-4	(4-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone	1345966-78-0	C$_{21}$H$_{23}$NO$_2$
RCS-4 ortho isomer Synonym: RCS-4 2-methoxy isomer	(2-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone	not available	C$_{21}$H$_{23}$NO$_2$
RCS-4 butyl homologue	(4-methoxyphenyl)(1-butyl-1*H*-indol-3-yl)methanone	not available	C$_{20}$H$_{21}$NO$_2$
WIN 48,098 Synonym: Pravadoline	(4-methoxyphenyl)[(2-methyl)-1-[2-(morpholin-4-yl)ethyl]-1*H*-indol-3-yl]methanone	92623-83-1	C$_{23}$H$_{26}$N$_2$O$_3$

(d) Naphthylmethylindoles

No compounds have been seized yet in this category.

(e) Cyclopropoylindoles
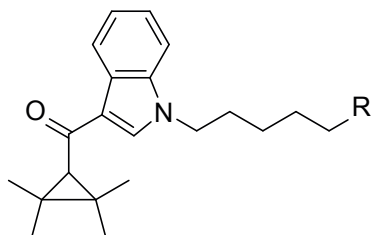
R=H

UR-144

R=F

XLR-11

Name	Chemical name	CAS No	Molecular formula
UR-144 *Synonym:* KM-X1	(2,2,3,3-tetramethylcyclopropyl)(1-pentyl-1*H*-indol-3-yl)methanone	1199943-44-6	$C_{21}H_{29}NO$
XLR-11 *Synonyms:* 5-FUR-144, 5-fluoro UR-144	(2,2,3,3-tetramethylcyclopropyl)(1-(5-fluoropentyl)-1*H*-indol-3-yl)methanone	1364933-54-9	$C_{21}H_{28}FNO$

(f) Adamantoylindoles
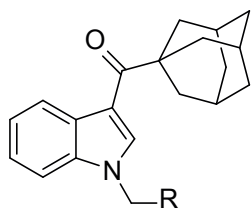
R=butyl

AB-001

R=1-methylpiperidin-2-yl

AM-1248

Name	Chemical name	CAS No	Molecular formula
AB-001 *Synonym:* JWH-018 (adamantyl)	(1-adamantyl)(1-pentyl-1*H*-indol-3-yl)methanone	1345973-49-0	$C_{24}H_{31}NO$
AM-1248	(1-adamantyl)[1-[(1-methylpiperidin-2-yl)methyl]-1*H*-indol-3-yl]methanone	335160-66-2	$C_{26}H_{34}N_2O$

(g) Indole carboxamides
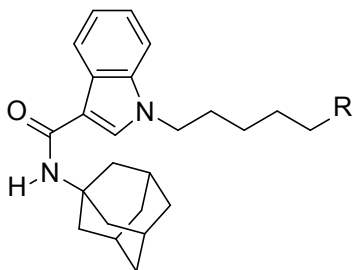
R=H

APICA

R=F

STS-135

Name	Chemical name	CAS No	Molecular formula
APICA *Synonyms:* 2NE1; JWH 018 adamantyl carboxamide	*N*-(1-adamantyl)-1-pentyl-1*H*-indol-3-carboxamide	1345973-50-3	$C_{24}H_{32}N_2O$
STS-135 *Synonym:* 5-fluoro APICA	*N*-(1-adamantyl)-1-(5-fluoropentyl)-1*H*-indol-3-carboxamide	1354631-26-7	$C_{24}H_{31}FN_2O$

3.5   Eicosanoids
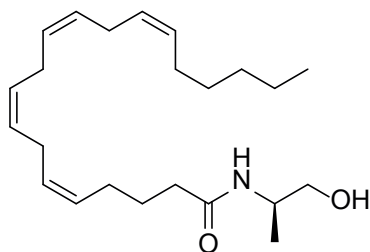
AM-356

Name	Chemical name	CAS No	Molecular formula
AM-356 *Synonym:* Methanandamide	*N*-(2-hydroxy-1*R*-methylethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide	157182-49-5	$C_{23}H_{39}NO_2$

3.6   Others

Name	Chemical name	CAS No	Molecular formula
APINACA *Synonym:* AKB48	*N*-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide	1345973-53-6	$C_{23}H_{31}N_3O$
CRA-13 *Synonyms:* CB-13; SAB-378	(naphthalen-1-yl)(4-pentyloxynaphthalen-1-yl)methanone	432047-72-8	$C_{26}H_{24}O_2$
JWH-307	(naphthalen-1-yl)(5-(2-fluorophenyl)-1-pentyl-1*H*-pyrrol-3-yl)methanone	914458-26-7	$C_{26}H_{24}FNO$
JWH-370	(naphthalen-1-yl)[5-(2-methylphenyl)-1-pentyl-1*H*-pyrrol-3-yl]methanone	914458-22-3	$C_{27}H_{27}NO$
Org 27569	5-chloro-3-ethyl-1*H*-indol-2-carboxylic acid [2-(4-piperidin-1-ylphenyl)ethyl]amide	868273-06-7	$C_{24}H_{28}ClN_3O$
Org 27759	5-fluoro-3-ethyl-1*H*-indol-2-carboxylic acid [2-(4-dimethylaminophenyl)ethyl]amide	868273-09-0	$C_{21}H_{24}FN_3O$
Org 29647	5-chloro-3-ethyl-1*H*-indol-2-carboxylic acid (1-benzylpyrrolidin-3-yl)amide	not available	$C_{22}H_{24}ClN_3O$
WIN-55,212-2	(naphthalen-1-yl)[(3*R*)-2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]methanone	131543-23-2	$C_{27}H_{26}N_2O_3$

4. Production and diversion

4.1 Synthesis of pure compounds

Aminoalkylindoles are by far the most prevalent compounds found in herbal products laced with synthetic cannabinoids. This is due to the fact that syntheses of aminoalkylindoles are less elaborate and complicated than syntheses of classical, non-classical or hybrid cannabinoids. In general, aminoalkylindoles can be synthesized without sophisticated laboratory equipment using inexpensive reagents and chemicals. However, there are a few exceptions where the compounds carry uncommon substituents such as adamantyl, tetramethylcyclopropyl and methyl piperidine derivatives which may be harder to synthesize and purify.

Common precursors for synthesis of aminoalkylindoles, which is usually carried out by Friedel-Crafts acylation at C3 followed by N-alkylation of a (substituted) indole or vice versa, are:

1. 1-alkylindoles and 1-alkyl-2-methylindoles (alkyl: butyl, pentyl, hexyl or others, halogenated if applicable)

2. 1-naphthoyl chlorides (e.g. substituted at C4)

One example of a synthetic route for naphthoylindoles such as JWH-073, JWH-073 (4-methylnaphthyl), JWH-018 and JWH-122 [25] is shown below:

Figure I. Example of a synthetic route for selected naphthoylindoles

R_1=n-C_4H_9	R_2=H	**JWH-073**
R_1=n-C_4H_9	R_2=CH_3	**JWH-073 (4-methylnaphthyl)**
R_1=n-C_5H_{11}	R_2=H	**JWH-018**
R_1=n-C_5H_{11}	R_2=CH_3	**JWH-122**
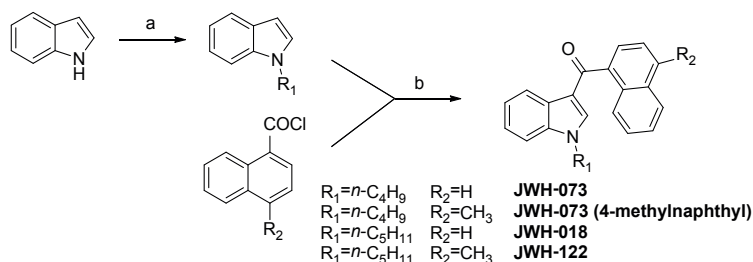
a Potassium tert-butoxide, butyl iodide or pentyl bromide, THF, RT.
b AlCl$_3$, DCM, 0°C.

For cyclohexylphenols of the CP-47,497-type, commonly obtainable precursors such as (3-(benzyloxy)phenyl) acetonitrile and cyclohex-2-en-1-one are required. It should to be noted that alternative approaches for synthesis are possible.

4.2   Production of herbal preparations

Although synthetic cannabinoids can generally be administered as pure substances, end products are usually designed for smoking. Most of these end products are made of herbal material laced with one or more synthetic cannabinoids and natural/artificial flavourings.

The mixing of the plant material with synthetic cannabinoids could be performed by putting the plant material in a cement mixer and adding a solution of synthetic cannabinoids in an organic solvent (e.g. acetone) to soak the material. After drying, the cannabinoids are distributed more or less homogeneously on the plant material. In many cases, traces of synthetic cannabinoids beside the main compounds could be detected in the end products. This could be a consequence of the mixing vessel not being cleaned thoroughly after each production cycle and hence leading to cross contamination. Sometimes crystalline powder is visible at the bottom of the packets, possibly from simple mixing of the plant material with the drugs in powder form, and resulting in an inhomogeneous mixture of the active compounds and the plant material.

4.3   Precursors and sources

Some of the synthetic cannabinoids present in these products can be bought from specialized chemical companies, but the prices for these high purity chemicals may be too expensive for use in herbal preparations. Cheaper alternatives are provided by many companies often located in Asia, although sources in Europe have been reported.

The quality of these compounds in general does not meet pharmaceutical standards and they are often contaminated with synthetic by-products and derivatives originating from inefficient synthetic processes [26]. However in some cases, seizures in kilogram quantities were found to be very pure, but also smaller amounts may be of high purity [24]. To mislead customs authorities, these products are usually shipped using wrong declarations, e.g. "polyphosphate", "maleic acid", "fluorescent whitening agent", "ethyl vanillin", "cotton", "paper sample", "TiO_2" (titanium dioxide) or "fish tank cleaner".

4.4   Typical seized materials

The most prevalent forms of seized products are ready-to-smoke mixtures of plant material laced with synthetic cannabinoid additives. They often contain more than

one active compound, rising to about six in the same product. This is followed by seized products containing pure substances in powder form. These products are usually used for large-scale production of herbal preparations or by the end users who would concoct their own blend of herbal mixtures. Products resembling hashish in their appearance are not so commonly encountered.

4.5   Adulterants/masking agents

In the first generation of herbal products, adulterants such as tocopherols or oleamide were frequently added [1]. It remains unclear if the purpose was to mask the active ingredients or if they were added as preservatives. Tocopherol acts as an antioxidant and was mainly found in products containing CP-47,497-C8. Oleamide on the other hand exhibits cannabis-like behavioural responses when ingested and may have been added to modify the psychotropic effects. These additives are no longer present in current products. However, many products still contain natural/artificial flavourings such as ethylvanillin, eugenol or other terpenoids [27]. It is unlikely that these compounds have any significant impact on the pharmacological activity of the products.

5. Qualitative and quantitative analysis of materials containing synthetic cannabinoids

Generally, in attempting to establish the identity of a controlled drug in suspect material, the analytical approach must entail the determination of at least two uncorrelated parameters, one of which should provide information on the chemical structure of the analyte (for example, IR, MS; or tandem methods such as GC-MS).

It is recognized that the selection of these parameters in any particular case would take into account the drug involved and the laboratory resources available to the analyst. It is also accepted that unique requirements in different jurisdictions may dictate the actual practices followed by a particular laboratory.

5.1 General aspects

As synthetic cannabinoids are often found as additives to herbal mixtures, the strategy for analysis would be different to some extent from the analysis of classical herbal drugs such as cannabis or drugs in other forms such as heroin, cocaine and amphetamine-type-stimulants. Some important aspects of analysis that should be considered are summarized as follows:

Table 1. Important aspects of analysis for consideration

Analytical aspects	Considerations
Sampling	• The herbal products could be grouped according to brand names and packaging for sampling. However, within the same group, it is also possible to have dissimilar contents • Packets would need to be opened for visual inspection of the plant material
Homogeneity	• Inhomogeneous distribution may be possible depending on the method of application of the synthetic cannabinoids onto the herbal material • Effective homogenization or sampling strategy required for quantitative analysis

Table 1.   Important aspects of analysis for consideration *(continued)*

Extraction	• Straightforward extraction procedures could be used for chromatographic analysis as active substances are typically laced onto the surface of the plant material
	• Extraction would not be required for ion mobility spectrometric (IMS) or ambient mass spectrometric (MS) techniques such as direct analysis in real time mass spectrometry (DART-MS) and desorption electrospray ionization mass spectrometry (DESI-MS)
Sensitivity	• Sensitive methods are required as synthetic cannabinoids are present in low concentrations (typically 1-30 mg/g) and interferences from matrix may be possible
	• Presumptive tests such as colour tests would not be appropriate
Variety of synthetic cannabinoids	• The number and type of substances vary considerably from sample to sample
	• Reference spectrum libraries would need to be constantly updated to keep up with the vast variety of substances available
	• Availability of reference samples would pose an issue as not all types of synthetic cannabinoids could be procured
	• When a new unknown compound is encountered, a general approach towards isolation and chemical characterization of the new compound is described in chapter 7

Qualitative analysis may be performed by TLC, IMS, IR, GC-FID, GC-IRD or GC-MS. GC-MS can be regarded as the gold standard, as it provides not only excellent chromatographic resolution but also in general allows for identification of active ingredients by their EI-MS spectra. But GC-MS may have its limit analysing regioisomers. To distinguish these, additional measurements with other analytical techniques have to be done for unequivocal identification of the correct regioisomer (e.g. IR or GC-IRD).

TLC is an inexpensive and rapid technique which allows processing of high numbers of samples and thus, can serve to significantly reduce the number of required GC-MS analyses. By coupling TLC with ambient mass spectrometric techniques such as DESI-MS, identification of a broad range of analytes can be achieved. As for IMS, it can be regarded as a sensitive screening method as other presumptive tests such as colour tests and microcrystal tests are not suitable to analyse herbal products.

As for solid material containing pure substances, IR techniques may be applied. Mobile FTIR systems are also useful for rapid screening of seized materials in the field suspected to contain pure synthetic cannabinoids in powder form. If there is only a single synthetic cannabinoid in the seized sample, identification of the

compound by IR is also possible with extracts of herbal mixtures after evaporation of the solvent on the ATR diamond cell.

For quantitative analyses, GC-FID, HPLC (or UHPLC) and LC-MS (or LC-MS/MS) methods can be used. Liquid chromatographic methods may be superior to gas chromatographic methods in cases of the presence of high amounts of fatty acid derivatives, which might cause interferences in gas chromatographic methods.

The recommended minimum guidelines for method selection have been formulated by the Scientific Working Group on Drugs (SWGDRUG) and available online at this website: http://www.swgdrug.org/.

5.2   Sampling

The principal reason for a sampling procedure is to permit an accurate and mean-ingful chemical analysis. Because most methods–qualitative and quantitative–used in forensic drug analysis laboratories require very small aliquots of material, it is vital that these small aliquots be representative of the bulk from which they have been drawn. Sampling should conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or by regional or international organizations. For general aspects of representative drug sampling of multi-unit samples, refer to the *Guidelines on Representative Drug Sampling* (http://www.unodc.org/unodc/en/scientists/publications_manuals.html). For seized material with obvious external characteristics, a sampling method based on the Bayes' model may be preferred over the hypergeometric approach.

The use of an approved sampling system also helps to preserve valuable resources and time by reducing the number of determinations needed. It is recognized that there may be situations where, for legal reasons, the normal rules of sampling and homogenization cannot be followed.

With herbal mixtures, modified sampling strategies may be required, particularly in cases whereby a large variety of different brands are encountered in the same seizure. It should be noted that the content of a particular brand of product could change over time as well. If a large number of identical products or bulk material is seized, commonly used sampling strategies may be applied.

5.3   Extraction and sample preparation

Qualitative analysis

Add 1 ml of medium-polar or non-polar solvents such as methanol, ethanol, aceto-nitrile, ethyl acetate, acetone or isooctane to a small portion of sample (e.g. 100 mg of plant material or 1-2 mg of solid material). Sonicate the extract and filter or centrifuge, if necessary, before analysis.

Quantitative analysis

Pulverize and homogenize the plant/solid materials before taking samples for analysis. Homogenization can also be performed in an electric grinder or deep frozen with liquid nitrogen in a mortar. Homogenization of only an aliquot of the sample should be avoided, as the cannabinoids tend to settle down at the bottom of a sample. At least two individual samples should be generated from the homogenate depending on homogeneity and mass of the original material.

Extract the samples using medium-polar or non-polar solvents such as methanol, ethanol, acetonitrile, ethyl acetate, acetone or isooctane. Sonicate the mixture for more effective extraction and filter before analysis. For better recovery efficiency, the number of extractions performed could be increased. Soxleth extraction may also be used although this might be too elaborate for routine use in forensic laboratories.

5.4   Analysis of synthetic cannabinoids

5.4.1   Presumptive tests

Presumptive tests such as colour tests and microcrystal tests would not be appropriate due to low concentrations of the analytes in the herbal mixtures and possible interferences by the sample matrix. Although there are some commercially available presumptive tests for a few specific synthetic cannabinoids, there are currently no presumptive tests which cover the whole range of synthetic cannabinoids.

5.4.2   Thin-layer chromatography (TLC)

TLC is a commonly used technique for the separation and detection of illicitly manufactured drugs. It is inexpensive, rapid and flexible in the selection of both the stationary and mobile phase and amenable to a wide variety of substances, in base and salt form, ranging from most polar to non-polar materials. As the TLC plates are discarded after analysis, problems due to contamination of the stationary phase by matrix compounds (e.g. fatty acid derivatives), which are frequently observed for HPLC columns, would not arise.

Classical and non-classical cannabinoids (e.g. HU-210 and CP-47,497-C8) can be selectively and sensitively detected with UV light, Fast Blue RR reagent, iodine as well as iodoplatinate whereas the aminoalkylindoles (e.g. JWH-018, JWH-081, JWH-210) can be detected with UV light, iodine or iodoplatinate.

TLC plates (stationary phases)

Coating: Silica gel G with layer thickness of 0.25 mm and containing an inert indicator, which fluoresces under UV light wavelength 254 nm (Silica gel GF254).

Typical plate sizes: 20x20 cm; 20x10 cm; 10x5 cm (the latter should be used with the 10 cm side vertical with the TLC tank).

Plates prepared by the analyst must be activated before use by placing them into an oven at 120°C for at least 10 to 30 min. Plates are then stored in a grease-free desiccator over orange silica gel*. Heat activation is not required for commercially available coated plates.

Methods

Developing solvent systems

Prepare a developing solvent system (system A, B or C as shown in the table below) as accurately as possible by use of pipettes, dispensers and measuring cylinders. Leave the solvent system in the TLC tank for a time sufficient to allow vapour phase saturation to be achieved prior to the analysis (with adsorbent paper-lined tanks, this takes approximately 5 min).

Table 2.   Developing solvent systems for TLC

System	Solvents	Solvent proportions (by volume)
System A	*n*-Hexane	2
	Diethylether	1
System B [28]	Toluene	9
	Diethylamine	1
System C [28]	Ethyl acetate	18.5
	Methylene chloride	18
	Methanol	3
	Concentrated NH_4OH	1

Preparation of sample solutions

As the purpose of the TLC assay of herbal products is qualitative analysis, homogenization of the herbal material is not necessary. To a suitable amount of herbal mixture, e.g. 100 mg, extract with approximately 10-fold amount of solvent under ultrasonication for at least 10 min and subsequently centrifuge the mixture. Suitable solvents are acetonitrile (well defined sample spots observed) or methanol (better solvent for synthetic cannabinoids but less well defined sample spots observed).

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*Blue silica gel can also be used. However, due care should be taken as blue silica gel contains cobalt (II) chloride which is possibly carcinogenic to humans.

Preparation of standard solutions

Standard solutions are prepared at a concentration of 0.5 mg/ml in a suitable solvent.

Spotting and developing

Apply as separate spots 1 µl and 5 µl aliquots of sample solution, 2 µl of the standard solutions and 2 µl of solvent (as a negative control) on the TLC plate. Spotting must be done carefully to avoid damaging the surface of the plate.

Analytical notes

- The starting point of the run i.e. the "spotting line" should be 2 cm from the bottom of the plate.

- The spacing between applications of sample (spotting points) should be at least 1 cm and spots should not be placed closer than 1.5 cm to the side edge of the plate.

- To avoid diffuse spots during development, the size of the sample spot should be as small as possible (2 mm) by applying solutions in aliquots rather than a single discharge.

- Allow spots to dry and place plate into solvent-saturated tank (saturation of the vapour phase is achieved by using solvent-saturated pads or filter paper as lining of the tank).

- Remove plate from the development tank as soon as possible as the solvent reaches the development line (10 cm from starting line) marked beforehand; otherwise, diffused spots will occur.

Visualization/detection

The plates must be dried prior to visualization. This can be done at room temperature or by use of a drying box, oven or hot air. In the latter cases, care must be taken that no component of interest is subject to thermal decomposition.

Visualization/detection methods

(a)   UV light at 254 nm

Dark spots against a green background are observed. The spots are marked and if necessary, a digital photograph recorded.

(b)   Freshly prepared Fast Blue RR reagent

Dissolve 0.10 g of Fast Blue RR in 10 ml of distilled water and add 4 ml of 20 % (w/v) sodium hydroxide solution. The classical or non-classical cannabinoids appear as orange-reddish spots when the plate is sprayed with the reagent. If necessary, the plate is photographed after drying for documentation.

(c) Iodine

Place the dried plate in a TLC chamber containing solid iodine crystals. The synthetic cannabinoids appear as yellow to brown spots. If necessary, the plate is photographed for documentation.

(d) Iodoplatinate

Dissolve 5 g of chloroplatinic acid hexahydrate and 35 g of potassium iodide in 1650 ml of distilled water. Then, add 49.5 ml of concentrated hydrochloric acid. The synthetic cannabiniods appear as green/yellow, white/pink or purple spots. If necessary, the plate is photographed after drying for documentation.

Interpretation

After visualization, mark spots (e.g. by pencil) and calculate retardation factor (R_f) values.

$$R_f \;=\; \frac{\text{Migration distance: from origin to centre of spot}}{\text{Development distance: from origin to solvent front}}$$

Results

R_f values for selected synthetic cannabinoids using the above methods are as follows:

Table 3. TLC R_f values for selected synthetic cannabinoids using various developing systems

Compound	System A	R_f values System B	System C
Org 29647	0.00	—	—
AM-1220	0.00	—	—
AM-2233	0.00	—	—
Org 27759	0.01	—	—
Org 27569	0.01	—	—
JWH-200	0.02	0.60	0.85
HU-210	0.05	0.34	0.78
RCS-4 ortho isomer	0.16	—	—
RCS-4	0.18	0.67	0.87
AM-2201	0.18	0.75	0.82
AM-694	0.18	—	—

Compound	System A	R_f values System B	System C
JWH-015	0.22	0.73	0.91
JWH-018	0.25	0.76	0.91
JWH-250	0.26	0.74	0.91
JWH-072	0.31	—	—
JWH-007	0.31	—	—
JWH-307	0.35	—	—
JWH-073	0.36	0.75	0.91
JWH-251	0.36	0.71	0.88
JWH-203	0.40	—	—
JWH-081	0.41	0.71	0.88
JWH-122	0.41	—	—
JWH-019	0.42	0.76	0.91
JWH-020	0.44	—	—
JWH-412	0.44	—	—
JWH-210	0.45	0.75	0.85
JWH-398	—	0.71	0.88
CP-47,497	—	0.31	0.77
CP-47,497-C8	—	0.31	0.77
CP-55,940	—	0.14	0.52
RCS-8	—	0.70	0.88
WIN-55,212-2	—	0.58	0.86

Due to the similarity of R_f values for some compounds, it is recommended that another method with more distinguishing power (e.g. GC-MS, GC-IRD) be used to confirm these substances.

Analytical notes

- R_f values are not always reproducible due to small changes in plate composition and activation, in solvent systems, tank saturation or development distance. Therefore, the R_f values provided are indications of the chromatographic behaviour of the substances listed.

- It is essential that reference standards be run simultaneously on the same plate.

- For identification purposes, both the R_f value and the colour of the spots after spraying with the appropriate visualization reagents should always be considered.

5.4.3   Ion mobility spectrometry (IMS)

IMS is a fast and sensitive technique that is suitable for the detection of trace organics under atmospheric pressure conditions. It can be used as a rapid screening technique for many drugs of abuse including synthetic cannabinoids. IMS allows for easy sampling and handling by touching the surface of the herbal mixture with a wooden rod and transferring the adherent particles distributed over the surface onto a Teflon filter for analysis. As portable IMS systems are commercially available, IMS can be used as a rapid detection technique in the field (e.g. crime scene investigations).

IMS can be operated in positive and negative ion modes. Aminoalkylindoles can be detected in positive ion mode while non-classical cannabinoids (e.g. CP-47,497-C8) can be detected in negative ion mode. Typical plant matrices and aromatic components of the herbal mixtures do not interfere with IMS signals of the active substances present.

Although IMS has limited selectivity, a new aminoalkylindole will give a signal in the typical detection window for aminoalkylindoles of the IMS plasmagram and hence, subsequent confirmatory analysis with more sophisticated instrumentation should be carried out.

The following steps are part of a field-tested and fit-for-purpose IMS method for portable IMS systems:

IMS operating conditions (positive ion mode)

Ionization source:	^{63}Ni beta-emitting source or x-ray tube
Desorber temp.:	290°C
Inlet temp.:	285°C
Drift tube temp.:	235°C
Drift flow:	300 ml/min
Sample flow:	200 ml/min
Stand-by flow:	51 ml/min
Drift gas:	Dried, purified air
Carrier gas:	Dried, purified air
Calibrant/reactant:	Nicotinamide
Calibrant temp.:	80°C
Gate width:	200 µs
Desorption time:	8.0 s
Scan period:	20 ms
Number of scans:	20
Drift tube length:	6.9 cm
Threshold:	50 d.u. (for JWH-018)
FWHM:	400 µs (for JWH-018)

Note: The above conditions may be altered as long as appropriate validation is carried out.

As there is a higher prevalence of the aminoalkylindoles in herbal products, the IMS is typically operated in positive ion mode. For switching to the negative ion mode, some of the parameters listed above have to be modified (e.g. desorber temp.: 222°C, inlet temp.: 238°C, drift tube temp.: 105°C).

Procedures

For analysis of herbal mixtures, touch the sample surface with a wooden rod. Take care that no visible particles of the plant material are on the rod after sampling. Sweep the tip of the rod several times over the Teflon filter placed in the IMS system and start analysis. To account for inhomogeneity, multiple sampling with the wooden rod is recommended.

Results

Aminoalkylindoles give sharp signals in positive ion mode within a characteristic detection window at high drift times and can be matched to reference substances by their reduced ion mobilities (K_0). Non-classical cannabinoids (e.g. CP-47,497 and its homologues) can be detected with lower but sufficient sensitivity in negative ion mode within a characteristic detection window distant from the detection window for the explosives. K_0 values for selected synthetic cannabinoids using the above method are as follows:

Table 4. IMS K_0 values for selected synthetic cannabinoids

Compound	K_0 values (positive ion mode) [cm²/(V*s)]	K_0 values (negative ion mode) [cm²/(V*s)]
JWH-210	0.9596	—
JWH-081	0.9720	—
AM-1220	0.9878	—
JWH-019	0.9915	—
JWH-200	0.9926	—
JWH-122	0.9950	—
AM-2201	1.0163	—
JWH-250	1.0263	—
JWH-018	1.0288	—
AM-694	1.0348	—
JWH-203	1.0455	—
JWH-251	1.0483	—
JWH-073	1.0658	—
RCS-4	1.0659	—
CP-55,940	—	0.9045
CP-47,497-C8	—	0.9185
CP-47,497	—	0.9354

Typically, substances that exhibit differences in their K_0 values < 0.025 can not be discriminated by IMS (e.g. JWH-019/JWH-200 or JWH-073/RCS-4). As this method is only suitable as a rapid screening technique, it is recommended that another method with more distinguishing power (e.g. GC-MS, GC-IRD) to be used to confirm these substances.

Analytical notes

- The IMS system must be allowed to warm up for at least 30 min before analysis to yield stable drift times.

- For system verification, a reference standard mixture (usually supplied by the instrument manufacturer) covering the largest portion of the relevant drift time scale should be analysed and appropriate alarms should be created by comparison with the reference data in the library.

- Before any sample analysis, the Teflon sample filter has to be subjected to a blank measurement to exclude contamination.

- Pure samples of all aminoalkylindoles of interest should be analysed and the resulting reduced ion mobilities stored in the library.

- If the signal of the internal calibrant is completely suppressed, the analysis should be repeated with a smaller amount of sample.

- Monitoring of the signal intensity over desorption time can additionally help to avoid false positives.

5.4.4   Gas chromatography-mass spectrometry (GC-MS)

GC-MS is one of the most commonly used techniques for the identification of forensic drug samples. As a hyphenated technique, it unifies the separation power and sensitivity of a GC with the analyte specificity of a spectroscopic technique. It can provide high specific spectral data on individual compounds in a complex mixture without prior isolation.

Sample preparation and extraction procedure

Add 1 ml of medium-polar or non-polar solvents such as methanol, ethanol, acetonitrile, ethyl acetate, acetone or isooctane to a small portion of sample (e.g. 100 mg of plant material or 1-2 mg of solid material). Sonicate the extract and filter before analysis.

Preparation of internal standard solution (for retention locking if required)

Dissolve *N,N*-dibenzyl-2-chlorobenzamide in methanol to give a concentration of 20 μg/ml. Add an aliquot of the internal standard to the sample/standard solution if retention time locking of the analysis is required.

Preparation of standard solutions

Prepare a standard solution of synthetic cannabinoid at a concentration of 1 mg/ml with an appropriate solvent (e.g. methanol, ethanol, acetonitrile, ethyl acetate, acetone or isooctane).

GC-MS operating conditions

GC oven conditions:	Column temp. initially set at 240°C and held isothermal for 1 min immediately after injection and ramped to 330°C at a rate of 6°C/min with a final isotherm of 4 min
Column:	TG-SQC, TG-5MS, DB-5MS or equivalent, 30 m x 0.25 mm i.d., 0.25 μm film thickness
Inlet:	Mode: splitless (purge flow 30 ml/min at 0.3 min) Temp.: 250°C Carrier gas: Helium, 1 ml/min, constant flow Injection volume: 1 μl
Detector:	Ionization mode: EI mode, 70 eV Transfer line temp.: 280°C Ion source temp.: 225°C
MS parameters:	Solvent delay: 3 min Scan mode Scanning mass range: 30 – 600 amu at 2.17 scan/sec

Note: The above conditions may be altered as long as appropriate validation is carried out.

Results

GC retention times (RT) for selected synthetic cannabinoids using the above operating conditions are as follows:

Table 5. GC retention times and major GCMS ions for selected synthetic cannabinoids

Compound	GC RT (mins)	Major GCMS ions (m/z)
UR-144	6.05	214, 144, 296, 311M$^+$
XLR-11	6.70	232, 144, 314, 329M$^+$
CP-47,497	6.80	215, 233, 318M$^+$, 300
CP-47,497-C8 (1S/3S or 1R/3R)	7.40	215, 233, 332M$^+$, 314
CP-47,497-C8 (1S/3R or 1R/3S)	7.65	215, 233, 332M$^+$, 314
Internal standard	8.10	139, 141, 244, 335M$^+$
RCS-4 ortho isomer	8.75	321M$^+$, 264, 304, 144
JWH-251	9.20	214, 144, 116, 319M$^+$
JWH-203	10.00	214, 144, 116, 339M$^+$
JWH-250	10.15	214, 144, 116, 335M$^+$
RCS-4	10.65	321M$^+$, 264, 135, 214
JWH-015	11.35	327M$^+$, 326, 310, 270
JWH-073	11.78	327M$^+$, 200, 284, 310
AM-694	11.82	232, 435M$^+$, 220, 360
APINACA	11.90	215, 145, 294, 365M$^+$
JWH-412	12.15	359M$^+$, 302, 145, 173
Org 27759	12.50	147, 134, 118, 353M$^+$
JWH-018	12.60	341M$^+$, 284, 324, 214
JWH-007	13.00	355M$^+$, 354, 340, 298
JWH-307	13.15	385M$^+$, 155, 188, 314
JWH-019	13.45	355M$^+$, 284, 228, 338
AM-2201	13.70	359M$^+$, 232, 284, 342
JWH-122	13.90	355M$^+$, 298, 338, 214
JWH-210	14.50	369M$^+$, 312, 352, 214
MAM-2201	14.80	373M$^+$, 298, 356, 232
Org 29647	15.05	159, 91, 143, 381M$^+$
JWH-081	15.30	371M$^+$, 314, 354, 214
AM-1248	15.60	98, 70, 99, 390M$^+$
AM-2232	16.20	225, 352M$^+$, 127, 284
AM-1220	16.30	98, 127, 155, 382M$^+$
JWH-200	16.75	100, 127, 155, 384M$^+$
Org 27569	19.30	187, 174, 253, 409M$^+$

Note: M$^+$ refers to molecular ion

Identification is accomplished by comparing the retention time and mass spectrum of the analyte with that of a reference standard. All compounds identified by GC-MS ideally should be compared to a current mass spectrum of the appropriate reference standard, preferably obtained from the same instrument, operated under the same conditions. In view of the difficulty of obtaining reference samples of synthetic cannabinoids, care must be taken in the use of reference spectra obtained from other sources such as commercial libraries or user generated spectra.

For the correct identification of regioisomers, additional techniques such as IR, GC-IRD or MS[n] might be necessary.

5.4.5   Gas chromatography (GC) with flame ionization detection (GC-FID) [25]

GC-FID could be employed for both qualitative and quantitative determinations. The method for the quantitative GC-FID analysis of a few selective synthetic cannabinoids is described here to be used as a guide for adaptation and modification which would be required for other synthetic cannabinoids of interest. It is good to note that for samples with very low concentrations, it would be more advisable to employ a more sensitive technique, e.g. LC-MS or LC-MS/MS for quantitative determinations.

Preparation of internal standard (IS) solution

Dissolve methyl oleate in methanol to give a concentration of 0.8 mg/ml.

Preparation of synthetic cannabinoid standard solutions

Prepare accurately standard solutions of targeted synthetic cannabinoid in an appropriate working concentration range. This method could be validated for the concentration range of 0.02-2.00 mg/ml in methanol. Usually at least five standard solutions should be prepared for a good linear calibration curve. Then, add 500 μl of the internal standard solution to 500 μl of each standard solution and vortex the mixture. Inject 1 μl of the mixture into the gas chromatograph.

Preparation of sample solutions (unknown "herbal mixture")

Obtain a representative sample from the seized material. Homogenize and accurately weigh 50 mg of seized material into a centrifugation tube and add quantitatively 5 ml of methanol. Sonicate and centrifuge the mixture for 5 min at 2,500 rpm. Then, add 500 μl of the internal standard solution to 500 μl of the supernatant solution and vortex the mixture. Inject 1 μl of the mixture into the gas chromatograph. At least one duplicate analysis should be carried out.

GC operating conditions

Detector: FID

Column: Factor Four VF-5ms containing 5 % phenyl methyl poly-
                    siloxane or equivalent, 30 m x 0.25 mm i.d., 0.25 μm film
                    thickness

Carrier gas: Helium 1.2 ml/min

Detector gas: Hydrogen 35 ml/min, air 350 ml/min

Inlet temp.:   250°C

Detector temp.:   280°C

Oven temp.:   Column temp. initially set at 70°C and ramped to 180°C
                    at a rate of 40°C/min and then ramped to 300°C at a
                    rate of 10°C/min

Injection volume: 1 μl

Split ratio: 30:1

Note: The above conditions may be altered as long as appropriate validation is carried
out.

Results

Elution order and the corresponding retention time are as follows:

Table 6. GC-FID elution order and corresponding retention times for selected synthetic cannabinoids

Compound	Retention time (min)
Internal standard	9.3
JWH-073	18.3
JWH-018	19.4
JWH-073 (4-methylnaphthyl)	20.1
JWH-122	22.8

Calculations

The percentage of targeted synthetic cannabinoid in the sample is then calculated by first plotting a linear calibration curve of the response ratio observed from the calibration standards (i.e. peak area of cannabinoid standard/peak area of IS) against concentration of cannabinoid standard used (mg/ml). From the response of the unknown sample solution and the corresponding value from the calibration curve,

the percentage of synthetic cannabinoid in the sample could be obtained using the formula below:

$$\% \text{ synthetic cannabinoid} = 100 \times \frac{V \times \dfrac{(R_s - b)}{a}}{W_S}$$

Where;

V:	Volume of extraction solvent used (ml)
R_S:	Response ratio observed for the sample (i.e. peak area of cannabinoid/peak area of IS)
a:	Gradient/slope of the calibration curve
b:	Intercept of the calibration curve
W_S:	Weight of the sample (mg)

Generally, with modern GC instrumentation and software, manual calculation of purity would not be required. Usually after input by the operator of the concentrations of the different calibration standards and the unknown sample solution, the calibration curve will be established and calculations will be performed automatically for any single point along the curve upon completion of the analytical run. Typically, the result will then be expressed as the percentage content of the unknown drug in the original sample material, i.e. as the sample purity (weight of the analyte relative to the sample weight).

5.4.6   Ultra high performance liquid chromatography (UHPLC)

UHPLC systems have enhanced chromatographic capabilities compared to traditional high performance liquid chromatography (HPLC) as operating pressures are higher and columns are packed with sub-2 μm particles giving rise to higher separation efficiency. The separation speed of the UHPLC is also significantly greater which allows for faster sample throughput. Furthermore, it is more environmentally friendly with lower solvent consumption and reduced waste disposal.

Since there is a large variety of stationary and mobile phases available to the analyst, one method for quantitative UHPLC analysis is described below and can be modified for improved performance. This method has been field-tested within forensic casework and is considered fit-for-purpose. With adequate verification and validation, the same method can also be extended to other synthetic cannabinoids.

Preparation of internal standard (IS) solution

Weigh 20 mg of 1-pyrenebutyric acid into a 10 ml volumetric flask and dilute to volume with methanol to give a concentration of 2.0 mg/ml.

Preparation of synthetic cannabinoid standard solutions

Accurately weigh 5 mg of analyte into a 5 ml volumetric flask and dilute to volume with methanol to give a stock solution with a concentration of 1.0 mg/ml. For some analytes (e.g. JWH-018, JWH-019 and JWH-073), solutions with 1.0 mg/ml concentrations are commercially available. The stock solution can be stored for at least one year with refrigeration.

Prepare accurately an appropriate working concentration range. Usually at least five standard solutions should be prepared for a good linear calibration curve. An example of the preparation of a 6-point calibration curve is given below:

Table 7. Example of the preparation of a 6-point calibration curve

Calibration level	Volume of standard stock solution added (µl)	Volume of IS solution added (µl)	Total volume after dilution with methanol (ml)	Final concentration of IS (µg/ml)	Final concentration of cannabinoids (µg/ml)
Level 1	10	40	10	8	1
Level 2	10	8	2	8	5
Level 3	25	4	1	8	25
Level 4	50	4	1	8	50
Level 5	37.5	2	0.5	8	75
Level 6	50	2	0.5	8	100

Preparation of sample solutions (unknown "herbal mixture")

Obtain a representative sample from the seized material and carefully homogenize. Accurately weigh 200 mg of the sample into a flask and add quantitatively 2 ml of methanol. Extract under sonication for 15 min, invert flask at least 10 times, and centrifuge for 2 min at 5,000 rpm, or allow to settle. Then, transfer the liquid to another flask and repeat extraction step twice with portions of 2 ml of methanol. Take an aliquot of approximately 2 ml of the combined extracts and filter using a syringe filter (≤ 0.45 µm). Then, accurately pipette 50 µl of the filtrate and 8 µl of IS solution into a 2 ml volumetric flask and dilute to volume with mobile phase A. Inject 5 µl of the sample solution into the UHPLC. At least one duplicate analysis should be carried out.

UHPLC operating conditions

Column:	Acquity UPLC BEH Phenyl, 100 mm x 2.1 mm i.d., 1.7 µm particle size
Mobile phase:	A: 95 % acetonitrile, 4.9 % water, 0.1 % formic acid
	B: 95 % water, 4.9 % acetonitrile, 0.1 % formic acid
Gradient:	0.0 – 12.5 min 41 % A
	12.5 – 20.0 min 50 % A
	20.0 – 23.0 min 60 % A
	23.0 – 27.5 min 41 % A
Flow rate:	0.4 ml/min
Pressure:	512 bar
Temp.:	30°C
Detection:	Photodiode Array (PDA), detection wavelengths (see below)
Injection volume:	5 µl

Note: The above conditions may be altered as long as appropriate validation is carried out.

Results

Identification is accomplished by comparing the retention time of the analyte with the retention time of a reference standard. The internal standard allows the use of retention index as an additional identification criterion. Furthermore, the UV spectrum of the analyte has to be compared with that of a reference standard solution.

Table 8. UHPLC retention times and detection wavelengths for selected synthetic cannabinoids

Compounds	Retention time (min)	Detection wavelength (nm)
JWH-200	1.9	217
AM-1220	2.3	217
Internal standard	5.7	198/242
AM-694	11.8	209
RCS-4	12.8	209
CP-47,497	13.7	198
JWH-250	15.5	209
JWH-073	16.3	217
CP-47,497-C8	16.6	198
JWH-251	17.0	209
JWH-203	17.6	209
JWH-018	19.2	217
JWH-007	20.0	217

Compounds	Retention time (min)	Detection wavelength (nm)
JWH-081	20.6	209
JWH-122	21.9	217
JWH-019	22.5	217
JWH-210	24.0	217

Quantitation

Due to possible matrix interactions, internal standard calibration is strongly advised. The use of peak area for quantitation is recommended because negative effects from peak broadening can be minimized. Previously characterized "herbal mixtures" or blends can be employed as precision controls.

Analytical notes

- The above method is suitable for "herbal mixtures" with cannabinoid contents of up to 100 mg/g, resulting in sample solutions with concentrations of up to 100 µg/ml. If the contents are found to be above 100 mg/g, then further dilution or repeated analysis with lesser sample is required.

- The same method could also be used for qualitative analyses, however it is not necessary for duplicate analysis. It is sufficient to analyse only one sample per homogenate with direct one-time extraction.

Calculations

The percentage of targeted synthetic cannabinoid in the sample is then calculated by first plotting a linear calibration curve of the response ratio observed from the calibration standards (i.e. peak area of cannabinoid standard/peak area of IS) against concentration of cannabinoid standard used (mg/ml). From the response of the unknown sample solution and the corresponding value from the calibration curve, the percentage of synthetic cannabinoid in the sample could be obtained using the formula below:

$$\% \text{ synthetic cannabinoid} = 100 \times \frac{V \times \frac{(R_S - b)}{a}}{W_S}$$

Where;

V: Volume of extraction solvent used (ml)

R_S: Response ratio observed for the sample (i.e. peak area of cannabinoid/peak area of IS)

a: Gradient/slope of the calibration curve

b: Intercept of the calibration curve

W_S: Weight of the sample (mg)

Generally, with modern LC instrumentation and software, manual calculation of purity would not be required. Usually after input by the operator of the concentrations of the different calibration standards and the unknown sample solution, the calibration curve will be established and calculations will be performed automatically for any single point along the curve upon completion of the analytical run. Typically, the result will then be expressed as the percentage content of the unknown drug in the original sample material, i.e. as the sample purity (weight of the analyte relative to the sample weight).

5.4.7   Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

LC-MS/MS is a powerful technique which combines the separation features of conventional HPLC or UHPLC with the detection capabilities of a tandem mass spectrometer, resulting in significantly increased selectivity and reduced interference between active ingredients and matrix. Its low limits of detection allow for trace analysis and the analysis of biological specimens such as blood and hair. With high sensitivity and selectivity, LC-MS/MS is suitable for both qualitative and quantitative analysis of low concentration synthetic cannabinoids in complex herbal mixtures.

One method for quantitative LC-MS/MS analysis is described below and can be modified for improved performance. This method has been field-tested within forensic casework and is considered fit-for-purpose. With adequate verification and validation, the same method can also be extended to other synthetic cannabinoids.

Preparation of internal standard (IS) solution

Weigh 200 mg of diphenylamine (DPA) into a 2 l volumetric flask and dilute to volume with ethanol to give a concentration of 100 mg/l.

Preparation of synthetic cannabinoid standard stock solution

Prepare a standard stock solution containing all analytes to be quantified (e.g. JWH-018, JWH-019 and JWH-073) in concentrations of 1.0 mg/l and the internal standard diphenylamine at a concentration of 100 μg/l as follows:

Accurately pipette 100 µl IS solution of 100 mg/l and 100 µl of 1 g/l solutions of each analyte (1 mg/ml concentrations are commercially available) into a 100 ml volumetric flask and dilute to volume with ethanol. The stock solution can be stored for at least one year with refrigeration.

Preparation of synthetic cannabinoid standard working solution

For making up the working standard solutions, the IS solution of 100 mg/l has to be first diluted 1,000 times to give a concentration of 100 µg/l (DIS solution). This solution is used to dilute the standard stock solution to the desired concentration.

Prepare accurately an appropriate working concentration range. Usually, at least five standard solutions should be prepared for a good linear calibration curve. An example of the preparation of a 5-point calibration curve is given below:

Table 9. Example of the preparation of a 5-point calibration curve

Calibration level	Volume of standard stock solution added (µl)	Volume of volumetric flask used dilute to volume with DIS solution (ml)	Final concentration of IS (µg/l)	Final concentration of cannabinoids (µg/l)
Level 1	30	10	100	3
Level 2	100	10	100	10
Level 3	300	10	100	30
Level 4	1 000	10	100	100
Level 5	2 000	10	100	200

Preparation of sample solutions (unknown "herbal mixture")

Obtain a representative sample from the seized material and carefully homogenize. Accurately weigh 100 mg of sample into a 50 ml volumetric flask and make up to the mark with IS solution (100 mg/l). Extract under sonication for 5 min, invert flask at least 10 times, and centrifuge for 2 min at 5,000 rpm, or allow to settle. Take an aliquot of approximately 2 ml and filter using a syringe filter (≤ 0.45 µm). Then, accurately pipette 50 µl of the filtrate into a 50 ml volumetric flask and dilute to volume with ethanol. Inject 5 µl of the sample solution into the LC-MS/MS. At least one duplicate analysis should be carried out.

LC-MS/MS operating conditions

LC:

Column:	C18 analytical column (e.g., 100 mm × 2.1 mm i.d., 3.5 μm), C18 guard column (10 mm × 2.1 mm i.d., 3.5 μm)
Mobile phase:	0.1% formic acid (A): water (B): methanol (C)
Gradient:	Initial A:B:C = 10:70:20, linear to 10:5:85 within 10 min, 10 min isocratic, back to initial conditions within 1 min, 4 min equilibration (total run time 25 min)
Flow rate:	0.2 ml/min
Column temp.:	30°C
Injection volume:	5 μl

MS/MS:

Detection mode :	Multiple reaction monitoring (MRM)
Ionization mode:	Simultaneous positive and negative electrospray ionization (ESI$^+$ and ESI$^-$)
Capillary voltage:	3.5 kV
Ion source temp.:	120°C
Desolvation temp.:	350°C
Cone gas:	Nitrogen, flow 60 l/h
Desolvation gas:	Nitrogen, flow 650 l/h
Collision gas:	Argon

Note: The above conditions may be altered as long as appropriate validation is carried out.

The following table show mass spectrometric data and parameters for some selected synthetic cannabinoids and the internal standard (DPA):

Table 10. LC-MS/MS mass spectrometric data and parameters for selected synthetic cannabinoids

Analyte	Ionization mode	Precursor ion (m/z)	Product ions (m/z)	Cone voltage (V)	Collision energy (eV)
DPA (IS)	ESI$^+$	170.17	93.26	31	28
JWH-018	ESI$^+$	342.20	154.99	30	25
			145.07		42

Analyte	Ionization mode	Precursor ion (m/z)	Product ions (m/z)	Cone voltage (V)	Collision energy (eV)
JWH-019	ESI$^+$	356.15	154.99	34	25
			126.99		44
JWH-073	ESI$^+$	328.10	155.12	33	22
			126.85		50
JWH-081	ESI$^+$	372.10	185.25	33	25
			214.29		25
JWH-122	ESI$^+$	356.35	169.43	29	25
			214.21		25
JWH-200	ESI$^+$	385.15	154.99	25	20
			114.25		25
JWH-210	ESI$^+$	370.25	183.46	33	26
			214.40		26
JWH-250	ESI$^+$	336.20	120.95	25	20
			188.19		16
AM-2201	ESI$^+$	360.10	155.37	30	25
			145.14		40
RCS-4	ESI$^+$	322.20	135.03	25	24
			76.74		50
CP-47,497	ESI$^-$	317.2	299.08	45	26
			159.59		55

Note: Precursor ions are detected as [M+H]$^+$ in ESI$^+$ mode or [M-H]$^-$ in ESI$^-$ mode.

Results

Identification is accomplished by comparing the retention time of the analyte with that of a reference standard solution. The internal standard allows the use of retention index as an additional identification criterion. Furthermore, the ratio of intensities of both mass transitions (precursor → product ion 1/precursor → product ion 2) of an analyte has to be compared with that of a reference standard solution. Appropriate mass transitions should be selected to avoid interference between different analytes, particularly in isomers (e.g. JWH-019 and JWH-122). Hence, even co-eluting compounds can be discriminated. In some cases, recording of the product spectrum of a particular precursor (Daughter Scan; DS) may be necessary for an unambiguous identification. Caution has to be applied when identifying regio-isomeric compounds.

Table 11.   LC-MS/MS retention times for selected synthetic cannabinoids

Compounds	Retention times (min)
JWH-200	11.7
Diphenylamine (IS)	15.0
AM-2201	16.2
RCS-4	17.0
JWH-250	17.1
JWH-073	17.2
JWH-018	18.1
JWH-081	18.5
JWH-019	18.9
JWH-122	19.0
CP-47,497 (ESI⁻ mode)	19.2
JWH-210	19.9

Quantitation

Due to possible matrix interactions and features specific to mass spectrometers, internal standard calibration is strongly advised and matrix effects have to be explored. The use of peak area for quantitation is recommended because negative effects from peak broadening can be minimized. Generally, the most intense mass transitions (primary trace; upper product ions in table 10) are usually utilized for quantitation, while less intense mass transitions (secondary trace; lower product ions in table 10) may be favoured when interferences exists. Co-eluting analytes can also be quantified simultaneously with this method. Previously characterized "herbal mixtures" or blends can be employed as precision controls.

Calculations

The percentage of targeted synthetic cannabinoid in the sample is then calculated by first plotting a linear calibration curve of the response ratio observed from the calibration standards (i.e. peak area of cannabinoid standard/peak area of IS) against concentration of cannabinoid standard used (mg/ml). From the response of the unknown sample solution and the corresponding value from the calibration curve, the percentage of synthetic cannabinoid in the sample could be obtained using the formula below:

$$\% \text{ synthetic cannabinoid} = 100 \times \frac{V \times \dfrac{(R_S - b)}{a}}{W_S}$$

Where;

V: Volume of extraction solvent used (ml)

R_S: Response ratio observed for the sample (i.e. peak area of cannabinoid/peak area of IS)

a: Gradient/slope of the calibration curve

b: Intercept of the calibration curve

W_S: Weight of the sample (mg)

Generally, with modern LC instrumentation and software, manual calculation of purity would not be required. Usually after input by the operator of the concentrations of the different calibration standards and the unknown sample solution, the calibration curve will be established and calculations will be performed automatically for any single point along the curve upon completion of the analytical run. Typically, the result will then be expressed as the percentage content of the unknown drug in the original sample material, i.e. as the sample purity (weight of the analyte relative to the sample weight).

Analytical notes

- The above method is suitable for "herbal mixtures" with cannabinoid contents of up to 100 mg/g, resulting in sample solutions with concentrations of up to 200 µg/l. If the contents are found to be above 100 mg/g, then further dilution or repeated analysis with lesser sample is required.

- The same method could also be used for qualitative analyses, however it is not necessary for duplicate analysis. It is sufficient to analyse only one sample per homogenate with direct one-time extraction. The method is not suitable for non-targeted analysis.

- With the method described, the cannabinoids JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, AM-2201, RCS-4 and CP-47,497 can be detected simultaneously.

- It should be noted that CP-47,497 is detected only in negative ionization mode, whereas the other analytes are ionized in positive mode.

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6. Additional analytical techniques for the analysis of synthetic cannabinoids

This section gives a brief overview of some additional techniques and approaches that can be applied to the analysis of synthetic cannabinoids in herbal products.

6.1 Infrared spectroscopy (ATR-IR and FTIR)

In general, without extraction, qualitative analysis of herbal mixtures by infrared spectroscopy is not possible due to the complex matrix and the comparatively low concentration of the synthetic cannabinoids present in the herbal products. However, as the synthetic cannabinoids are generally laced onto the herbal matrix, in most cases with an extraction step, it is possible to obtain a good IR spectrum after evaporating the extract directly on the ATR diamond cell. However, the correlation factors that are calculated by the software of the IR spectrometer for synthetic cannabinoids in extracts of herbal mixtures are slightly lower than for the pure substances. Hence, a plausibility check is inevitable (e.g. visual comparison of the reference spectrum of the pure cannabinoid vs. the spectrum of the analysed sample extract).

For powdery seizures of synthetic cannabinoids, qualitative infrared spectroscopic analysis is more straightforward. Infrared spectroscopy can also be a useful tool for identification of new substances [29]. For structure elucidation of unknown compounds, infrared spectroscopy would be very useful to differentiate isomers when it is not possible using ion trap techniques (MS^n).

6.2 Gas chromatography-infrared detection (GC-IRD)

A tandem GC-IRD technique combines the separation power of the GC with the molecular identification of the FTIR. As there exists many variants of synthetic cannabinoids, GC-IRD would be a valuable tool for confirming the identity of very similar molecules such as regioisomers, diastereomers and other isobaric molecules that display almost identically MS spectra.

6.3   Ambient ionization mass spectrometry

As synthetic cannabinoids are essentially laced onto herbal material, ambient ionization mass spectrometric techniques such as direct analysis in real time mass spectrometry (DART-MS) [30], desorption atmospheric pressure photoionization (DAPPI) [31] or desorption electrospray ionization mass spectrometry (DESI-MS) could be employed to sample these cannabinoids directly on the plant material without the need for extraction and sample preparation. DESI-MS could also be used in combination with TLC.

6.4   High resolution mass spectrometry (HRMS)

Besides identification by accurate mass measurements, HRMS could be used to determine the precise elemental compositions of new synthetic molecules, calculation of double bond equivalents as well as precise mass of the fragment ions. Furthermore, HRMS in conjunction with mass defect filtering enables non-targeted analysis of related compounds and analogues which could prove very useful in screening for synthetic cannabinoids [32-34].

6.5   Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS)

Another possibility for direct qualitative analysis of herbal mixtures is MALDI-TOF-MS. It offers a simple and rapid operation, allows for high throughput analysis and could be utilised as a 'front screening' of confiscated material [35].

6.6   Nuclear magnetic resonance (NMR) spectroscopy

The availability of a large number of structurally related synthetic cannabinoids, requires effective tools that provide the necessary structural information for their differentiation. NMR, i.e. ^{1}H NMR and ^{13}C NMR, enables identification as well as structure elucidation of unknown new synthetic cannabinoids. Two-dimensional NMR experiments such as H,H-COSY, H,H-NOESY, H,C-HSQC and H,C-HMBC could also be employed to provide definite proof of the structure. Furthermore, NMR could also be used for quantitative determinations. While being a powerful tool for the identification of analogues, the cost of NMR spectroscopy and the technical expertise required prevent its widespread application in routine analysis [5-7, 9, 17].

7. Isolation and chemical characterization of new synthetic cannabinoids

Due to the sheer number of emerging new synthetic cannabinoids, it is very likely for an analyst to encounter an unknown substance in a herbal product and suspect the presence of a new synthetic cannabinoid. However, identification of this unknown substance would prove to be difficult without commercially available reference standards, reference spectra as well as relevant literature and research. Hence, in order to identify this new substance, it has to be first isolated from the herbal mixture into a pure/enriched compound and then various analytical techniques could be employed to characterize this compound. Figure II below illustrates a general approach towards isolation and characterization of a new synthetic cannabinoid.

Isolation of a new compound

The first step would be to identify a suitable solvent to extract the targeted unknown cannabinoid (e.g. methanol, ethanol, acetonitrile, ethyl acetate, acetone or isooctane) from the herbal product. Extraction should be carried out with sonication and the extract filtered. Then, the extract should be subjected to preparative/flash chromatography (e.g. silica gel column, preparative LC or TLC) to obtain a fraction containing the targeted unknown cannabinoid. This fraction should show a single spot with a TLC analysis (visualization by UV light and/or other reagents, e.g. Fast Blue RR reagent, iodine, iodoplatinate). Then, the fraction containing the pure/enriched compound, should be concentrated and used for subsequent analysis aimed at characterizing the unknown cannabinoid.

## Characterization of a new compound

There is a variety of techniques available for characterization of an unknown cannabinoid. A combination of techniques such as HRMS and NMR is important for unambiguous structure elucidation. Other techniques such as IR and MS/MS may be useful to provide other structural information including differentiation between isomers or diastereomers.

With these techniques, the structure of the unknown cannabinoid could be deduced and based on this, a reference standard should be synthesized (as it is not available commercially). The synthesized reference standard should be analysed with the same techniques mentioned, under the same conditions. If the analysis of the synthesized

reference standard yields the same results, the deduced structure of the unknown cannabinoid could be confirmed. However with UV/VIS techniques, identical UV/VIS spectra of sample and standard do not confirm the identity of the compound. Contrary to this, dissimilar UV/VIS spectra are useful information that confirms that the compound is indeed different from the standard.

While it is not necessary to perform all the above analytical techniques for characterization, it is important to verify and confirm any interpretation with analysis of a synthesized standard and/or through peer review from a reputable laboratory. Collaboration with academia would also be useful as some sophisticated instrumentation (e.g. NMR, HRMS) is not commonly available for routine use in most of the forensic science laboratories.

Figure II. A schematic diagram illustrating the isolation and chemical characterization of new synthetic cannabinoids
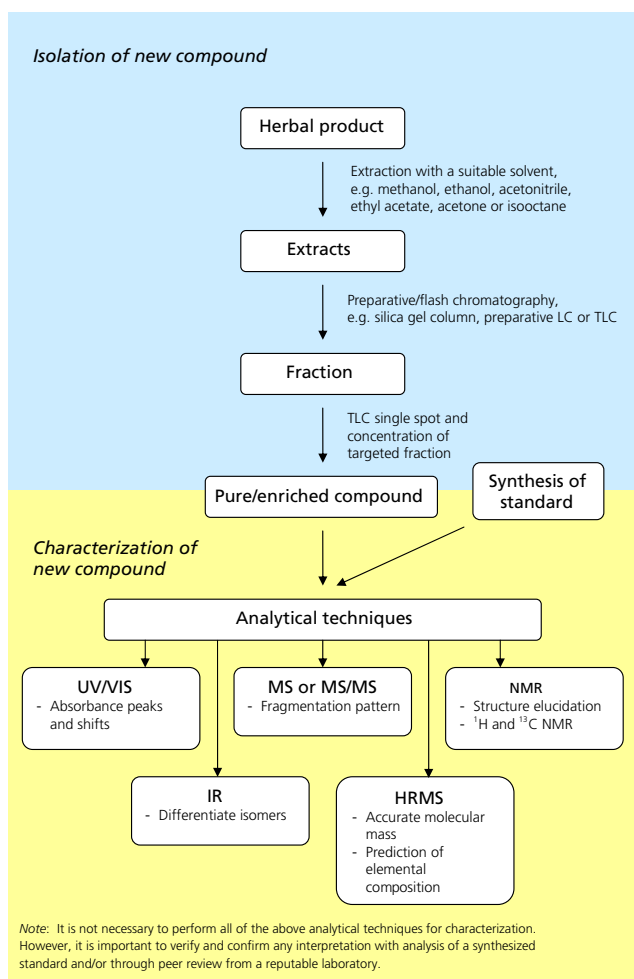
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