

Comprehensive review of the detection methods for synthetic cannabinoids and cathinones

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Received: 20 November 2014 / Accepted: 29 January 2015 / Published online: 6 March 2015
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Abstract A number of *N*-alkyl indole or indazole-3-carbonyl analogs, with modified chemical structures, are distributed throughout the world as synthetic cannabinoids. Like synthetic cannabinoids, cathinone analogs are also abused and cause serious problems worldwide. Acute deaths caused by overdoses of these drugs have been reported. Various analytical methods that can cope with the rapid changes in chemical structures are required for routine analysis and screening of these drugs in seized and biological materials for forensic and clinical purposes. Although many chromatographic methods to analyze each drug have been published, there are only a few articles summarizing these analytical methods. This review presents the various colorimetric detections, immunochemical assays, gas chromatographic–mass spectrometric methods, and liquid chromatographic–mass spectrometric methods proposed for the analysis of synthetic cannabinoids and cathinones.

Keywords Synthetic cannabinoids · Cannabimimetics · Cathinones · GC–MS–MS · LC–MS–MS · Analytical methods

Abbreviations

A-796260	[1-[2-(4-Morpholinyl)ethyl]-1 <i>H</i> -indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone
A-834735	[1-[(Tetrahydro-2 <i>H</i> -pyran-4-yl)methyl]-1 <i>H</i> -indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone
AB-001	Adamantan-1-yl(1-pentyl-1 <i>H</i> -indol-3-yl)methanone
AB-005	[1-[(1-Methyl-2-piperidinyl)methyl]-1 <i>H</i> -indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone
AB-CHMINACA	<i>N</i> -[(1 <i>S</i>)-1-(Aminocarbonyl)-2-methylpropyl]-1-(cyclohexylmethyl)-1 <i>H</i> -indazole-3-carboxamide
AB-FUBINACA	<i>N</i> -(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1 <i>H</i> -indazole-3-carboxamide
AB-PINACA	<i>N</i> -(1-Amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1 <i>H</i> -indazole-3-carboxamide
ADB-FUBINACA	<i>N</i> -(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1 <i>H</i> -indazole-3-carboxamide
ADBICA	<i>N</i> -(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1 <i>H</i> -indole-3-carboxamide
ADB-PINACA	<i>N</i> -(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1 <i>H</i> -indazole-3-carboxamide
AM-1220	[1-[(1-Methylpiperidin-2-yl)methyl]-1 <i>H</i> -indol-3-yl]-(naphthalen-1-yl)methanone

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AM-1248	Adamantan-1-yl[1-[(1-methyl-2-piperidinyl)methyl]-1 <i>H</i> -indol-3-yl]methanone	JWH-200	1-Naphthalenyl[1-[2-(4-morpholinyl)ethyl]-1 <i>H</i> -indol-3-yl]methanone
AM-1241	(2-Iodo-5-nitrophenyl)-[1-(1-methylpiperidin-2-ylmethyl)-1 <i>H</i> -indol-3-yl]methanone	JWH-203	2-(2-Chlorophenyl)-1-(1-pentyl-1 <i>H</i> -indol-3-yl)ethanone
AM-2201	[1-(5-Fluoropentyl)-1 <i>H</i> -indol-3-yl]-1-naphthalenylmethanone	JWH-250	2-(2-Methoxyphenyl)-1-(1-pentyl-1 <i>H</i> -indol-3-yl)ethanone
AM-2233	(2-Iodophenyl)[1-[(1-methyl-2-piperidinyl)methyl]-1 <i>H</i> -indol-3-yl]-methanone	JWH-251	2-(2-Methylphenyl)-1-(1-pentyl-1 <i>H</i> -indol-3-yl)ethanone
AM-679	(2-Iodophenyl)(1-pentyl-1 <i>H</i> -indol-3-yl)methanone	JWH-307	[5-(2-Fluorophenyl)-1-pentyl-1 <i>H</i> -pyrrol-3-yl](naphthalene-1-yl)methanone
AM-694	1-[(5-Fluoropentyl)-1 <i>H</i> -indol-3-yl]-(2-iodophenyl)methanone	LC	Liquid chromatography
AMB	Methyl (1-pentyl-1 <i>H</i> -indazole-3-carbonyl)-L-valinate	LC-MS	Liquid chromatography–mass spectrometry
APICA	<i>N</i> -(1-Adamantyl)-1-pentyl-1 <i>H</i> -indole-3-carboxamide	LC-MS-MS	Liquid chromatography–tandem mass spectrometry
APINACA	<i>N</i> -(1-Adamantyl)-1-pentyl-1 <i>H</i> -indazole-3-carboxamide	LLE	Liquid–liquid extraction
Cathinone	2-Amino-1-phenylpropan-1-one	LOD	Limit of detection
CI	Chemical ionization	LOQ	Limit of quantification
EI	Electron ionization	MAM-2201	[1-(5-Fluoropentyl)-1 <i>H</i> -indol-3-yl](4-methyl-1-naphthalenyl)methanone
ELISA	Enzyme-linked immunosorbent assay	MDPBP	3',4'-Methylenedioxy- α -pyrrolidinobutiophenone
ESI	Electrospray ionization	MDPPP	3',4'-Methylenedioxy- α -pyrrolidinopropiophenone
FDU-PB-22	Naphthalen-1-yl 1-(4-fluorobenzyl)-1 <i>H</i> -indole-3-carboxylate	MDPV	3,4-Methylenedioxypyrovalerone
5F-PB-22	1-(5-Fluoropentyl)-8-quinolinyl ester-1 <i>H</i> -indole-3-carboxylic acid	MN-18	<i>N</i> -1-Naphthalenyl-1-pentyl-1 <i>H</i> -indazole-3-carboxamide
FUB-PB-22	Quinolin-8-yl-1-(4-fluorobenzyl)-1 <i>H</i> -indole-3-carboxylate	MOPPP	4'-Methoxy- α -pyrrolidinopropiophenone
GC	Gas chromatography	NM-2201	Naphthalen-1-yl 1-(5-fluoropentyl)-1 <i>H</i> -indole-3-carboxylate
GC-MS	Gas chromatography–mass spectrometry	NMR	Nuclear magnetic resonance
GC-MS-MS	Gas chromatography–tandem mass spectrometry	NNEI	<i>N</i> -1-Naphthalenyl-1-pentyl-1 <i>H</i> -indole-3-carboxamide
HU-210	3-(1,1'-Dimethylheptyl)-6 <i>aR</i> ,7,10,10 <i>aR</i> -tetrahydro-1-hydroxy-6,6-dimethyl-6 <i>H</i> -dibenzo[<i>b,d</i>]pyran-9-methanol	MPBP	4'-Methyl- α -pyrrolidinobutiophenone
JWH-015	1-Naphthalenyl(2-methyl-1-propyl-1 <i>H</i> -indol-3-yl)methanone	MPHP	4'-Methyl- α -pyrrolidinohexanophenone
JWH-018	1-Naphthalenyl(1-pentyl-1 <i>H</i> -indol-3-yl)methanone	MPPP	4'-Methyl- α -pyrrolidinopropiophenone
JWH-019	1-Naphthalenyl(1-hexyl-1 <i>H</i> -indol-3-yl)methanone	MRM	Multiple reaction monitoring
JWH-030	1-Naphthalenyl(1-pentyl-1 <i>H</i> -pyrrol-3-yl)methanone	MS	Mass spectrometry
JWH-073	1-Naphthalenyl(1-butyl-1 <i>H</i> -indol-3-yl)methanone	MS-MS	Tandem mass spectrometry
		NPB-22	8-Quinolinyl 1-pentyl-1 <i>H</i> -indazole-3-carboxylate
		α -PBP	α -Pyrrolidinobutiophenone
		α -PHP	α -Pyrrolidinohexanophenone
		PP	Protein precipitation
		α -PPP	α -Pyrrolidinopropiophenone
		PTFE	Polytetrafluoroethylene

PV8	1-Phenyl-2-(pyrrolidin-1-yl)heptan-1-one
PV9	1-Phenyl-2-(pyrrolidin-1-yl)octan-1-one
α -PVP	1-Phenyl-2-(pyrrolidin-1-yl)pentan-1-one, α -pyrrolidinovalerophenone
PX1	(<i>S</i>)- <i>N</i> -(1-Amino-1-oxo-3-phenylpropan-2-yl)-1-(5-fluoropentyl)-1 <i>H</i> -indole-3-carboxamide
QUPIC	Quinolin-8-yl 1-pentyl-1 <i>H</i> -indole-3-carboxylate
QUCHIC	Quinolin-8-yl 1-(cyclohexylmethyl)-1 <i>H</i> -indole-3-carboxylate
RCS-4	(4-Methoxyphenyl)(1-pentyl-1 <i>H</i> -indol-3-yl)methanone
SDB-005	Naphthalen-1-yl 1-pentyl-1 <i>H</i> -indazole-3-carboxylate
SIM	Selected ion monitoring
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SRM	Selected reaction monitoring
THJ-018	1-Naphthalenyl(1-pentyl-1 <i>H</i> -indazol-3-yl)methanone
THJ-2201	[1-(5-Fluoropentyl)-1 <i>H</i> -indazol-3-yl](naphthalen-1-yl)methanone
TLC	Thin-layer chromatography
TOFMS	Time-of-flight mass spectrometry
UV	Ultraviolet
UR-144	(1-Pentyl-1 <i>H</i> -indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone
XLR-11	[1-(5-Fluoropentyl)-1 <i>H</i> -indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone
XLR-12	(2,2,3,3-Tetramethylcyclopropyl)[1-(4,4,4-trifluorobutyl)-1 <i>H</i> -indol-3-yl]methanone

Introduction

Currently, many illegal drugs are abused worldwide, with serious social problems arising as a consequence. Although various stimulants and narcotics have been in use to date, new drugs targeting cannabinoid receptors have been abused since their existence in herbal mixtures was disclosed in 2008 [1]. HU-210, a synthetic classical cannabinoid, and cyclohexylphenols were commonly used as recreational drugs, but mainstream use has since changed to *N*-alkyl indole-3-carbonyl derivatives, such as drugs of the JWH and AM series (Fig. 1), because their activities are stronger than those of the conventional cannabinoids. These compounds are called cannabimimetics or synthetic

cannabinoids and can be purchased as “spice” or “K2” in the drug market or via the Internet. Cathinones, also known as “bath salts” or “plant food,” are psychoactive drugs and are also abused as recreational drugs. The parent compound, cathinone, is a well-known stimulant, and can be isolated from the khat plant or produced by synthetic means. Cathinone analogs with high selectivity and strong activity for serotonin receptors and monoamine transporters have been distributed in the drug market (Fig. 2). The prevalence of cannabinoid and cathinone abuse in many countries has been reviewed elsewhere [2–7].

Although the same substances are distributed throughout the world, the times at which they are abused tend to vary depending on whether the substances are controlled by local laws. As shown in the reviews [2–7], new analogs appear in the drug market just after the preceding drug comes under regulation. Although many such substances are controlled in countries throughout the world, the regulations are usually limited by the structures of the drugs. Therefore, when the structure of a side chain or substitution is slightly different from that of the regulated drug, the analog is regarded as being beyond the scope of the regulation. These emerging drugs always show psychoactive actions because their chemical structures are similar to those of the drugs being controlled. However, the detailed pharmacological activities of these analogs are not known, which makes access easy and use of these drugs very dangerous to human health.

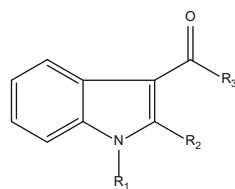
Although many researchers have focused on the development of detection methods, only a few analytical reviews that summarize the systematic identification and quantification techniques for these drugs have appeared [8–10]. In this review, we summarize the various techniques for the detection of synthetic cannabinoids and cathinones that have been published up to 2014, including colorimetric, immunochemical, and chromatographic methods.

Synthetic cannabinoids

Colorimetric detection

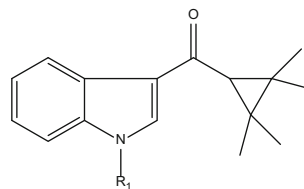
The Duquenois–Levine color test, which is used to identify classical cannabinoids such as Δ^9 -tetrahydrocannabinol, is negative for the synthetic cannabimimetics. The van Urk color test, which is used to identify indole-containing drugs of abuse, is also negative for these compounds. The use of 2,4-dinitrophenylhydrazine, which reacts with a keto moiety, is capable of reacting with synthetic cannabimimetics, such as the naphthoylindole, phenylacetylindole, benzoylindole, and cyclopropylindole classes, either in powder form or adsorbed onto plant material, and a positive test solution turns from yellow to orange. Although the LOD concentration was not detailed in the article, the solution

Indole 3-carbonyl derivatives



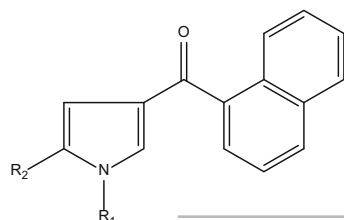
	R ₁	R ₂	R ₃
JWH-015	<i>n</i> -Propyl	Methyl	1-Naphthyl
JWH-018	<i>n</i> -Pentyl	H	1-Naphthyl
AM-1220	(1-Methyl-2-piperidiny)methyl	H	1-Naphthyl
JWH-200	2-(4-Morpholinyl)ethyl	H	1-Naphthyl
JWH-203	<i>n</i> -Pentyl	H	2-Chloro-benzyl
JWH-250	<i>n</i> -Pentyl	H	2-Methoxy-benzyl
JWH-251	<i>n</i> -Pentyl	H	2-Methoxy-benzyl
RCS-4	<i>n</i> -Pentyl	H	4-Methoxy-phenyl
AB-001	<i>n</i> -Pentyl	H	Adamantyl
AM-1248	(1-Methyl-2-piperidiny)methyl	H	Adamantyl
AM-679	<i>n</i> -Pentyl	H	2-Iodo-phenyl
AM-694	5-Fluoro- <i>n</i> -pentyl	H	2-Iodo-phenyl
AM-2233	(1-Methyl-2-piperidiny)methyl	H	2-Iodo-phenyl
AM-1241	(1-Methyl-2-piperidiny)methyl	H	2-Iodo-5-nitro-phenyl
- ^a	5-Fluoro- <i>n</i> -pentyl	H	3-Pyridinoyl

^a No abbreviated name available



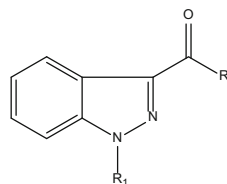
	R ₁
UR-144	<i>n</i> -Pentyl
XLR-11	5-Fluoro- <i>n</i> -pentyl
XLR-12	4,4,4-Trifluoro-butyl
AB-005	(1-Methyl-2-piperidiny)methyl
A-834735	(Tetrahydro-2 <i>H</i> -pyran-4-yl)methyl
A-796260	2-(4-Morpholinyl)ethyl

Pyrrole 3-carbonyl derivatives



	R ₁	R ₂
JWH-030	<i>n</i> -Pentyl	H
JWH-307	<i>n</i> -Pentyl	2-Fluoro-phenyl

Indazole 3-carbonyl derivative



	R ₁	R ₂
THJ-2201	5-Fluoro- <i>n</i> -pentyl	1-Naphthyl

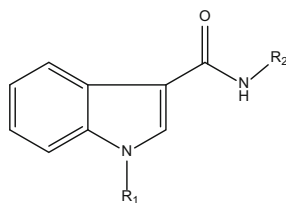
Fig. 1 Structures of synthetic cannabinoids

tested contained at least 10 mg of cannabimimetic powder suspended in methanol (1 ml) [11]. The Marquis reagent, which reacts with all nitrogen-containing drugs, is positive for cyclohexylphenols and the JWH series. Although Dragendorff reagent is also positive for the JWH series, its LOD concentration is higher than that of Marquis reagent. Fast blue BB reacts with cyclohexylphenols, and the LOD concentration is not lower than that of Marquis reagent [12]. Iodoplatinate is also used as a detection reagent after TLC [13]. Although it is possible to detect synthetic cannabinoids with each reagent in these screening tests, it is difficult to detect small amounts or mixtures of synthetic cannabinoids.

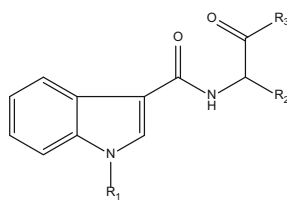
Immunochemical detection

ELISAs developed in-house could be calibrated at 5 ng/ml with the 5-OH and 4-OH metabolites of JWH-018 and JWH-250, respectively, and evaluated for the detection of synthetic cannabinoids in urine [14]. Recently, some commercially available immunoassay kits, such as Drug-Check K2/Spice Test, DrugSmart Cassette, and RapiCard InstaTest, have been developed for the detection of these drugs in urine. These devices are more useful than the colorimetric methods, because they do not require special reagents or tools, and the results are obtained easily and

Indole 3-carbonylamide derivatives

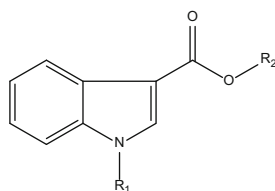


	R ₁	R ₂
APICA	<i>n</i> -Pentyl	Adamantyl
5F-APICA	5-Fluoro- <i>n</i> -pentyl	Adamantyl
NNEI(MN-24)	<i>n</i> -Pentyl	1-Naphthyl
5F-NNEI	5-Fluoro- <i>n</i> -pentyl	1-Naphthyl



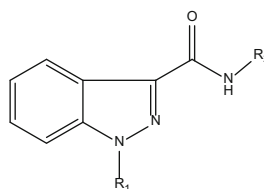
	R ₁	R ₂	R ₃
ADBICA	<i>n</i> -Pentyl	<i>t</i> -Butyl	NH ₂
PX1	5-Fluoro- <i>n</i> -pentyl	Benzyl	NH ₂

Indole 3-carbonylester derivatives

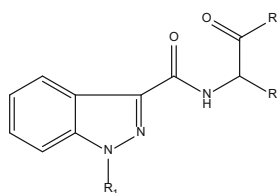


	R ₁	R ₂
NM-2201	5-Fluoro- <i>n</i> -pentyl	1-Naphthyl
FDU-PB-22	4-Fluoro-benzyl	1-Naphthyl
QUPIC(PB-22)	<i>n</i> -Pentyl	8-Quinoliny
5F-PB-22	5-Fluoro- <i>n</i> -pentyl	8-Quinoliny
QUCHIC(BB-22)	Cyclohexylmethyl	8-Quinoliny
FUB-PB-22	4-Fluoro-benzyl	8-Quinoliny

Indazole 3-carbonylamide derivatives

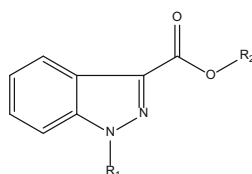


	R ₁	R ₂
APINACA	<i>n</i> -Pentyl	Adamantyl
5F-APINACA	5-Fluoro- <i>n</i> -pentyl	Adamantyl
MN-18	<i>n</i> -Pentyl	1-Naphthyl
5F-MN-18	5-Fluoro- <i>n</i> -pentyl	1-Naphthyl



	R ₁	R ₂	R ₃
AB-PINACA	<i>n</i> -Pentyl	<i>i</i> -Propyl	NH ₂
5F-AB-PINACA	5-Fluoro- <i>n</i> -pentyl	<i>i</i> -Propyl	NH ₂
ADB-PINACA	<i>n</i> -Pentyl	<i>t</i> -Butyl	NH ₂
AB-FUBINACA	4-Fluoro-benzyl	<i>i</i> -Propyl	NH ₂
ADB-FUBINACA	4-Fluoro-benzyl	<i>t</i> -Butyl	NH ₂
AB-CHMINACA	Cyclohexylmethyl	<i>i</i> -Propyl	NH ₂
AMB	<i>n</i> -Pentyl	<i>i</i> -Propyl	Methoxy
5F-AMB	5-Fluoro- <i>n</i> -pentyl	<i>i</i> -Propyl	Methoxy

Indazole 3-carbonylester derivatives



	R ₁	R ₂
5F-SDB-005	5-Fluoro- <i>n</i> -pentyl	1-Naphthyl
5F-NPB-22	5-Fluoro- <i>n</i> -pentyl	8-Quinoliny

Fig. 1 continued

quickly. The devices also can detect older types of synthetic cannabinoids, such as JWH-018 or JWH-073, but, unfortunately, new designer drugs such as QUPIC and AB-CHMINACA cannot be detected.

GC–MS detection

Typical mass spectra of synthetic cannabinoids are shown in Fig. 3. Molecular (M^+) and/or fragment ions observed

by full scan data acquisition of GC–MS reflect the structures of the synthetic cannabinoids [13, 15, 16]. As shown in Fig. 4, the fragmentation pathways of naphthoylindoles have been well studied for the identification of synthetic cannabinoids by GC–MS [12, 15]. Therefore, the identification of synthetic cannabinoids is facilitated by comparison of the spectra with commercial and open databases.

In naphthoylindoles, the carbonyl group fragment ions, which are caused by α -cleavage of the alkylamino group of



Name	R ₁	R ₂	R ₃	R ₄	R ₅
Cathinone	Methyl	H	H	H	H
Methcathinone	Methyl	Methyl	H	H	H
<i>N,N</i> -Dimethylcathinone	Methyl	Methyl	Methyl	H	H
Mephedrone	Methyl	Methyl	H	Methyl	H
3,4-Dimethylmethcathinone	Methyl	Methyl	H	Methyl	Methyl
4-Ethylmethcathinone	Methyl	Methyl	H	Ethyl	H
Methylone	Methyl	Methyl	H	Methylenedioxy	
Ethcathinone	Methyl	Ethyl	H	H	H
Ethylone	Methyl	Ethyl	H	Methylenedioxy	
Amfepramone	Methyl	Ethyl	Ethyl	H	H
Buphedrone	Ethyl	Methyl	H	H	H
4-Methylbuphedrone	Ethyl	Methyl	H	Methyl	H
Butylone	Ethyl	Methyl	H	Methylenedioxy	
<i>N</i> -Ethylbuphedrone	Ethyl	Ethyl	H	H	H
Eutylone	Ethyl	Ethyl	H	Methylenedioxy	
Pentedrone	Propyl	Methyl	H	H	H
α -Ethylaminopentiophenone	Propyl	Ethyl	H	H	H

Name	R ₁	R ₂	R ₃
α -PPP	Methyl	H	H
α -PBP	Ethyl	H	H
α -PVP	Propyl	H	H
α -PHP	Butyl	H	H
PV8	Pentyl	H	H
PV9	Hexyl	H	H
MPPP	Methyl	Methyl	H
MOPPP	Methyl	Methoxy	H
MPBP	Ethyl	Methyl	H
Pyrovalerone	Propyl	Methyl	H
Naphyrone	Propyl	Phenyl	
MPHP	Butyl	Methyl	H
MDPPP	Methyl	Methylenedioxy	
MDPBP	Ethyl	Methylenedioxy	
MDPV	Propyl	Methylenedioxy	

Fig. 2 Structures of cathinones

the indole, are typically observed. In addition, $[M-17]^+$ is certainly observed in naphthoylindoles. For example, fragment ions at m/z 284 and 214 are observed in JWH-018, corresponding to those of the indole moiety caused by α -cleavage of the *N*-pentyl of indole and naphthoyl. Fragment ions at m/z 127 and 155 are observed in JWH-018, corresponding to the naphthalene group caused by the α -cleavage of the carbonyl group. Moreover, ions at m/z 324 are observed as $[M-17]^+$ (Fig. 3a). Like naphthoylindoles, fragment ions caused by α -cleavage of the alkylamino group of the indole and carbonyl groups are shown, although $[M-17]^+$ is not observed for benzoylindoles. For example, fragment ions at m/z 264 and 214 are observed for RCS-4, caused by α -cleavage of *N*-pentyl of the indole and 4-methoxybenzoyl. The ions at m/z 127 and 155, which are caused by naphthyl and naphthoyl moieties of naphthoylindoles (Fig. 3a), and the ion at m/z 135 caused by the 4-methoxybenzoyl moiety (Fig. 3b) are useful as precursor ions for identification of these drugs by GC-MS-MS. On the other hand, the methylpiperidine moiety is bound to the nitrogen of the indole, and the ion at m/z 98 is observed as the base peak (Fig. 3d). Unlike naphthoyl and benzoyl indoles, the base peak of the

fragment ion caused by the *N*-alkylindole 3-carbonyl moiety for phenylacetyl (Fig. 3c), cyclopropyl, or adamantyl (Fig. 3f) indoles, is only shown in each full scan spectrum. Analogs, in which the indole skeleton is changed to an indazole, such as THJ-018, have also appeared on the market. In these analogs, molecular and *N*-dealkylated ions are typically observed in the spectrum (Fig. 3e).

Recently, amide- or ester-type analogs bonded with an *N*-alkylindole or *N*-alkylindazole 3-carbonyl moiety have appeared on the market [17, 18]. In these analogs, the abundance of the molecular ion is low, and the fragment ion caused by the indoyl (or indazoyl) moiety is observed as a base peak (Fig. 3f–k). Although the fragment ion caused by elimination of the terminal CO-NH_2 is lower than that of the cleavage of the amide moiety in indole analogs, such as ADBICA (Fig. 3h) [19], the fragment ion caused by elimination of terminal CO-NH_2 is as intense as that of the cleavage of the amide moiety in indazole analogs, such as ADB-PINACA and AB-CHMINACA (Fig. 3j, k) [20]. The substitution of the indole skeleton with the indazole moiety, such as in THJ-018 and THJ-2201 [21], has also been observed in these analogs. In these analogs, molecular and *N*-dealkylated ions are typically observed in the spectrum.

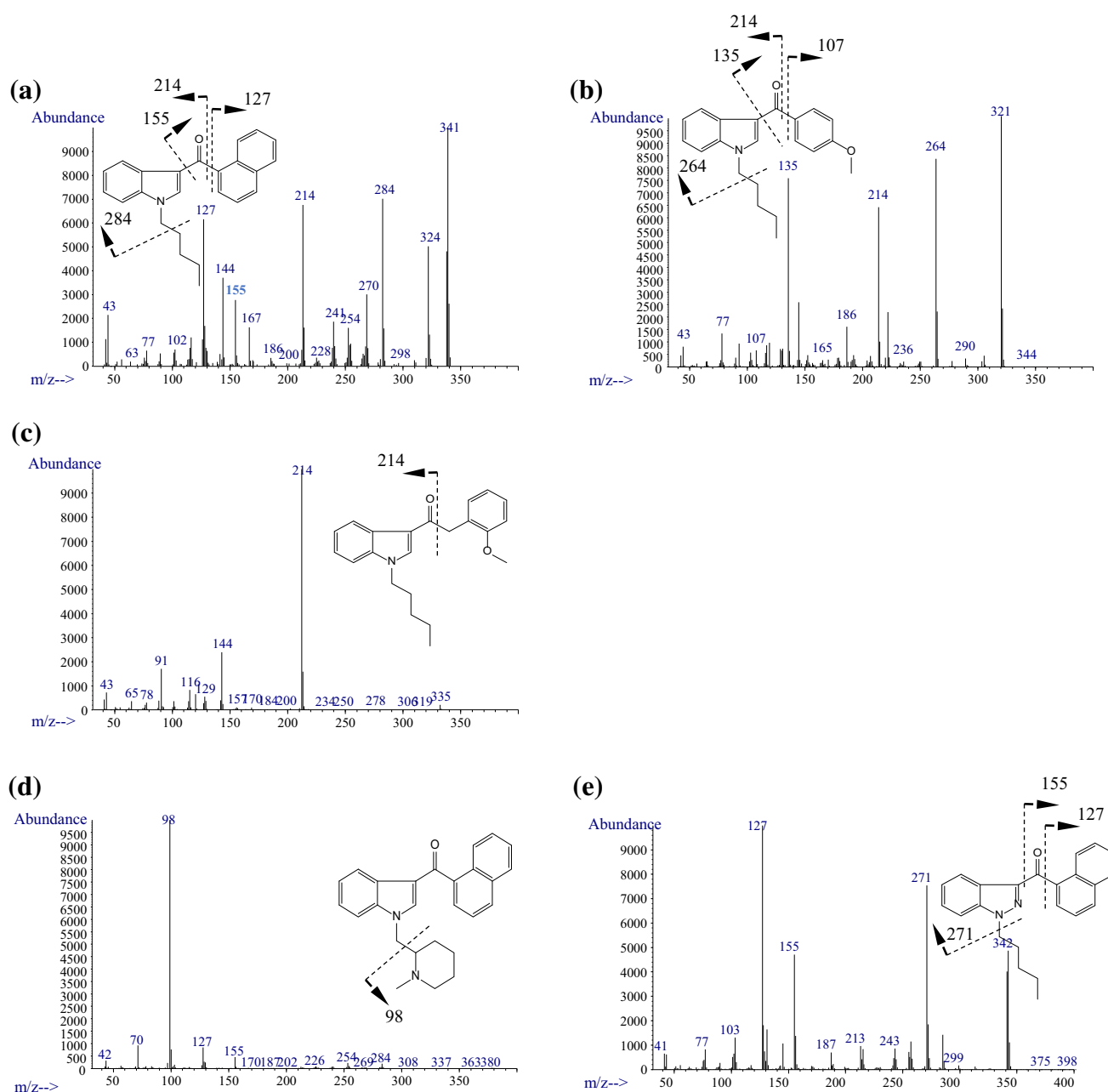


Fig. 3 Typical mass spectra of synthetic cannabinoids obtained by GC–MS. **a** JWH-018, **b** RCS-4, **c** JWH-250, **d** AM-1220, **e** THJ-018, **f** APICA, **g** NNEI, **h** ADBICA, **i** QUPIC (PB-22), **j** ADB-PINACA, **k** AB-CHMINACA

For example, in the simultaneous analysis of synthetic cannabinoid species, 10 mg of ground powder of the dried leaves was extracted with 10 ml of methanol under ultrasonication for 10 min. The extracts were centrifuged for 5 min at 3,000 rpm, and the supernatants were filtered and used for GC–MS analysis. The LODs were 0.5–1.0 mg/l, and linearity was obtained at concentrations up to 100 mg/l [16]. In another article [22], herbal samples (approximately 50 mg) were put into 10-ml headspace vials, and the vials were capped with 20-mm magnetic crimp seal caps with PTFE/silicone septa. The samples were incubated at 200 °C

with pulse-agitation at 250 rpm. A StableFlex carboxen/polydimethylsiloxane fiber was inserted into the headspace for 5 min for extraction. The fiber was then injected into the GC inlet for 15 min to desorb the analytes. The LOD of synthetic cannabinoid in the samples was at least 20 µg.

The tentative identification of synthetic cannabinoids appears easy, but similar mass spectra are sometimes obtained by GC–MS because regio- and ring-substituted analogs are still distributed on the market. The misidentification of these analogs arises when using only the information from the mass spectra. When tandem and high-resolution MS are

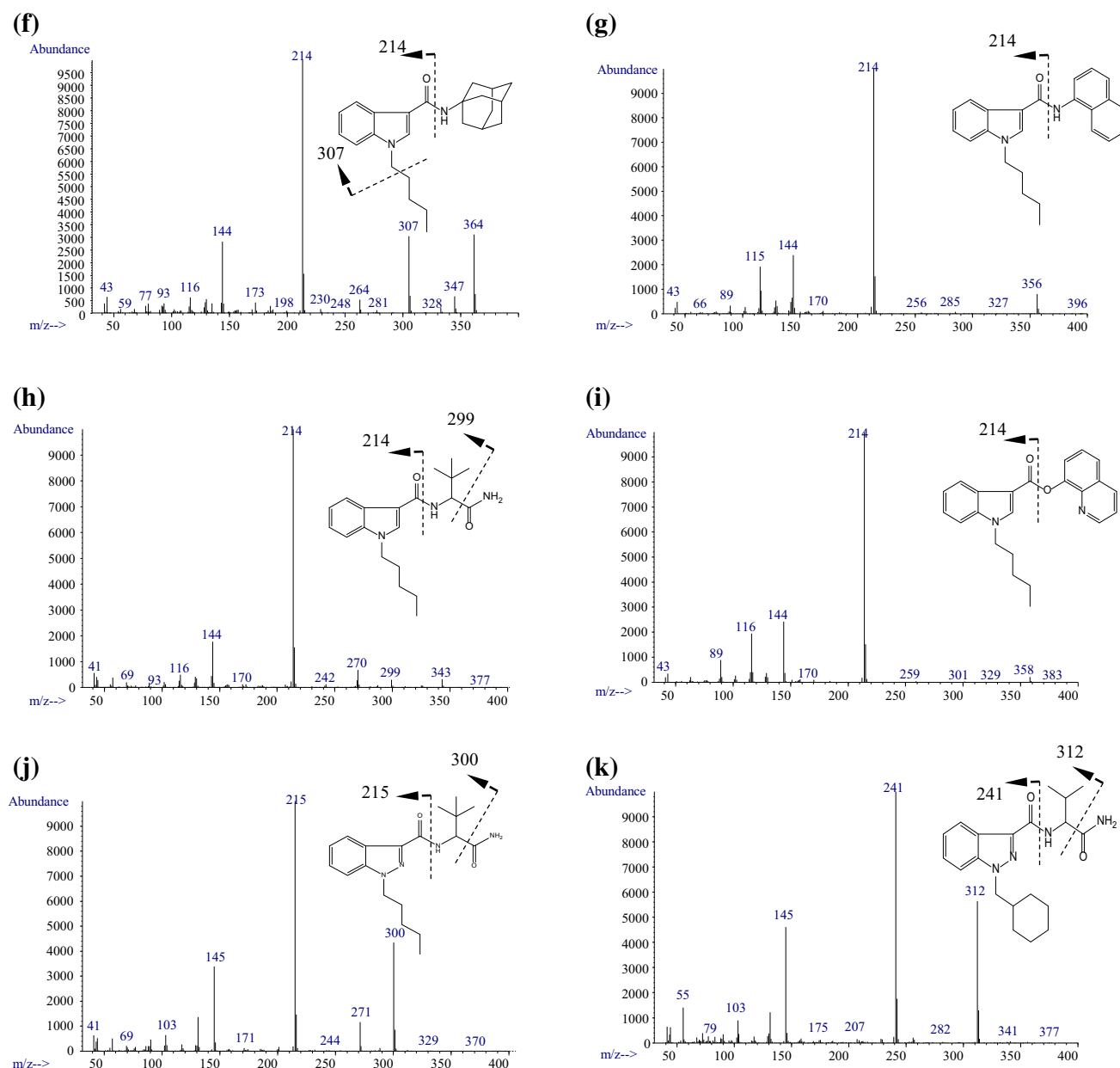


Fig. 3 continued

used to identify the conformational isomers or regioisomers, such misidentification does not occur [23–28]. Moreover, identification of cyclopropyl or ester analogs, such as UR-144 or QUPIC, is usually not possible because cyclopropyl analogs are heat-unstable and are easily degraded in the injection port of the GC instrument [29, 30].

LC–MS–MS detection

Many research groups have used LC–MS–MS for determination of synthetic cannabinoids in herbs and biological samples, and some have studied the fragmentation of

synthetic cannabinoids in detail [15, 31]. The probable fragmentation pathways are shown in Fig. 4. Because the protonated molecular ion is only observed by LC–MS, and the information acquired by LC–MS is lesser than that for GC–MS, it is necessary to obtain other data that reflect the chemical structures by LC–MS–MS or TOFMS. Fragment ions are observed by product ion scanning when the protonated molecular ion is used as the precursor ion. In naphthoylindole, ions at m/z 127 and 155 are generated by naphthyl and naphthoyl moieties. However, information about the indole moiety tends to be not revealed by LC–MS–MS. On the other hand, the *N*-alkyl moiety of a

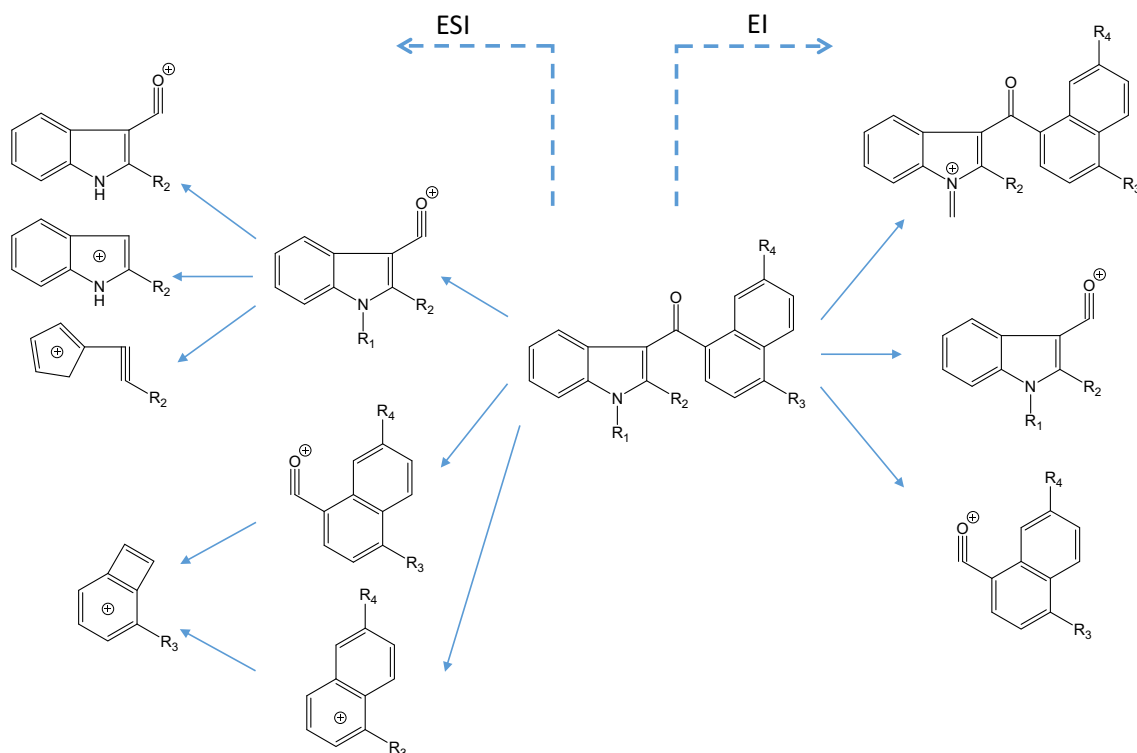


Fig. 4 Probable fragmentation pathways of synthetic cannabinoids by electrospray ionization and electron ionization (modified from references [12, 31])

synthetic cannabinoid is mainly modified for excretion into urine as a metabolite. Therefore, LC–MS–MS is a useful methodology to search for metabolites of synthetic cannabinoids in urine.

Recently, packages containing mixtures of multiple synthetic cannabinoids have been sold commercially, even though the package ingredients have been largely unknown to both sellers and buyers. In this aspect, the LC–MS–MS screening method is helpful in some estimation of the ingredients. Kneisel and Auwärter [32] demonstrated the simultaneous detection of 30 synthetic cannabinoids in serum; the LODs and LOQs were 0.01–2.0 and 0.1–2.0 ng/ml, respectively. There are many applications for analysis of synthetic cannabinoids in urine, hair, and oral fluids [33–37]. The typical published methods for analysis of synthetic cannabinoids in biological materials are summarized in Table 1 [38–54]. Simple LLE is usually used for the extraction of synthetic cannabinoids from biological materials because of the high hydrophobicity of the drugs. The chromatographic conditions are generally simple and do not require a special technique; octadecyl-type columns were used as analytical columns and analyses were performed in gradient mode.

The identification of an unknown drug in a biological material without information is almost always difficult, even if analysis is carried out with LC–MS–MS. To

overcome this situation, high-resolution MS or TOFMS become helpful tools for tentative estimation of parent drugs and their metabolites of synthetic cannabinoids.

To clarify the chemical structure of an unknown drug in a herbal blend product that contains more than several milligrams of the drug, GC–MS, LC–MS–MS, and high-resolution MS (or TOFMS) can be used to estimate the structure. The target compound is then purified by preparative LC or preparative TLC to obtain more than several milligrams of the compound of high purity, which is then analyzed by NMR spectroscopy [17–21]. The detailed chemical structure can be elucidated by the above laborious instrumental analyses.

Cathinones

Colorimetric detection

The Marquis reagent, which reacts with all nitrogen-containing drugs, is negative for cathinones, such as cathinone and mephedrone, but is positive for cathinone analogs that have a methylenedioxy moiety in each molecule. The cathinone analogs with a methylenedioxy moiety also react with the Chen reagent, which changes to orange in positive tests. Although the LOD concentration is not reported, the

Table 1 LC–MS or LC–MS–MS conditions for synthetic cannabinoids in biological materials

Target(s)	Sample(s)	Purification(s)	Column(s)	Mobile phase	LOD (ng/ml)	Linear range (ng/ml)	Reference(s)
JWH-018	Serum	LLE	Luna C18 (2) (150 mm, 2 mm ID, 5 µm) (Phenomenex)	10 mM ammonium acetate (0.1 % acetic acid, pH 3.2), methanol	0.07	0.21–20	[38]
JWH-018, JWH-073, JWH-019, JWH-250	Blood	LLE	Acquity UPLC HSS T3 (100 mm, 2.1 mm ID, 1.8 µm) (Waters)	1 % formic acid, methanol (1 % formic acid)	0.006–0.016	0.1–20	[39, 40]
Aminoalkylindoles, methanandamide	Serum	LLE	Luna phenyl hexyl (50 mm, 2 mm ID, 5 µm) (Phenomenex)	2 mM ammonium formate (0.2 % formic acid), methanol	0.1	0.1–2, 0.3–2 (methanandamide)	[41]
JWH-018, JWH-073, metabolites	Urine	Dilution (hydrolysis)	Zorbax Eclipse XDB-C18 (150 mm, 4.6 mm ID, 5 µm) (Agilent)	0.1 % formic acid, acetonitrile (0.1 % formic acid)	<2.0	2–100	[42]
Metabolites of JWH-018 and JWH-073	Urine	SPE (hydrolysis)	Zorbax Eclipse XDB-C18 (150 mm, 4.6 mm ID, 5 µm) (Agilent)	0.1 % formic acid/acetonitrile (0.1 % formic acid) (45:55), isocratic	<0.1	0.1–100	[43]
Metabolites of 8 synthetic cannabinoids	Urine	LLE (hydrolysis)	AQUASIL C18 (100 mm, 2.1 mm ID, 5 µm) (Thermo Scientific)	5 mM ammonium acetate, methanol/acetonitrile (1:1, 5 mM ammonium acetate)		0.1–10	[44]
Metabolites of JWH-018 and JWH-073	Urine	LLE (hydrolysis)	Acquity UPLC HSS T3 (100 mm, 2.1 mm ID, 1.8 µm) (Waters)	0.1 % formic acid (0.1 %), acetonitrile (0.1 % formic acid)		4–400	[45]
30 Synthetic cannabinoids	Serum	LLE	Luna phenyl hexyl (50 mm, 2 mm ID, 5 µm) (Phenomenex)	0.2 % formic acid (2 mM ammonium formate), methanol	0.01–2.0	0.1–2.0 (2–40 : JWH-387)	[32, 46–48]
Metabolites of 7 synthetic cannabinoids	Urine	LLE (hydrolysis)	Luna C18 (150 mm, 2 mm ID, 5 µm) (Phenomenex)	0.2 % formic acid (2 mM ammonium formate), methanol			[49]
22 Synthetic cannabinoids	Hair	Ethanol ext	Luna phenyl hexyl (50 mm, 2 mm ID, 5 µm) (Phenomenex)	0.2 % formic acid (2 mM ammonium formate), methanol	0.5 pg/mg		[37]
JWH-018, JWH-073	Blood	LLE	Acquity UPLC BEH C18 (50 mm, 2.1 mm ID, 1.8 µm) (Waters)	0.1 % formic acid, acetonitrile (0.1 % formic acid)	0.01	0.05–50	[50]
UR-144, metabolites, pyrolysis product	Urine	LLE (hydrolysis)	Zorbax Eclipse XDB-C18 (150 mm, 2.1 mm ID, 3.5 µm) (Agilent)	20 mM ammonium formate buffer (pH5), acetonitrile			[29]
UR-144, metabolites	Blood, urine	PP	Kinetex C18 (100 mm, 4.6 mm ID, 2.6 µm) (Phenomenex), Ascentis express C18 (7.5 cm, 2.1 mm ID, 2.7 µm) (Supelco)	0.1 % formic acid, acetonitrile (0.1 % formic acid)	0.15 (blood)	0.5–100 (blood)	[51]
9 Synthetic cannabinoids, 20 metabolites	Urine	PP (hydrolysis)	XB-C18 (50 mm, 3.0 mm ID, 2.6 µm) (Kinetex)	0.1 % formic acid, acetonitrile (0.1 % formic acid)	0.5–10		[34]

Table 1 continued

Target(s)	Sample(s)	Purification(s)	Column(s)	Mobile phase	LOD (ng/ml)	Linear range (ng/ml)	Reference(s)
MAM-2201	Blood	SPE	InertSustain C18 HP (100 mm, 3 mm ID, 3 μ m) (GL Sciences)	0.1 % acetic acid, acetonitrile	1	2.5–100	[52]
5F-PB-22	Blood, serum	LLE	Acquity UPLC BEH C18 (100 mm, 2.1 mm ID, 1.7 μ m) (Waters)	0.1 % formic acid, acetonitrile (0.1 % formic acid)	0.1	0.5–10	[53]
Indole derivative synthetic cannabinoids	Saliva, urine, blood	PP (blood) LLE (urine, saliva) (hydrolysis)	Ascentis C18 (150 mm, 2.1 mm, 5 μ m) (Supelco)	0.1 % formic acid (5 mM ammonium formate), acetonitrile (0.1 % formic acid)	0.1–0.5		[54]

described test solution contained cathinone powder (at least 10 mg) suspended in methanol (1 ml) [55–57]. The combination of Marquis, Ehrlich, Simon, Lieberman–Burehand, and Mandelin reagents is useful for the detection of cathinones in samples. Like synthetic cannabinoids, the identification of these compounds, of course, cannot be performed using these methods; moreover, the detection of small amounts or mixtures of cathinones is difficult.

Immunochemical detection

Some researchers have tried to detect cathinones in urine using immunoassay technology [58, 59]. Some articles revealed false-positive results by immunoassays; for example, MDPV was cross-reactive with phencyclidine [60]. Therefore, specific detection of cathinones by a commercial immunoassay is not yet possible.

GC–MS–MS detection

Mass spectral profiles of cathinones are very simple in the positive mode of GC–MS, because only the base peak originating from the immonium ion in each molecule is observed. The probable fragmentation pathways of cathinones are described in previous articles [61–64] and are shown in Fig. 5. However, this phenomenon makes the identification of cathinones difficult. To help identify cathinones, other information, such as tandem mass spectrometric data, are usually used because more structural information about the molecule is obtained.

Zuba [61] introduced the systematic identification of cathinones using the mass spectra obtained. First, it should be checked whether the molecular ion is observed. The immonium ion ($m/z = 16 + 14n$, $n = 1, 2, 3, \dots$) is then checked in the EI spectrum. If the immonium ion is found in the spectrum, the substance could be a straight-chained cathinone. If not, it is checked whether the ion for a pyrrolidine ring is observed ($m/z = 70 + 14n$, $n = 1, 2, 3, \dots$). If this ion is found in the spectrum, the substance could be a cathinone with a pyrrolidine ring in the molecule [61]. There are various regioisomers in cathinones. To identify the cathinones, it is necessary to assign both the location and length of the bonded alkyl chain. Moreover, the ring-substituted moiety is also needed to be assigned. Zuba [61] demonstrated the following rules: the fragment ions reflecting the ring-substituted moiety are observed at m/z 77 and 105 for a nonsubstituted phenyl ring, at m/z 91 and 119 for a methylphenyl ring, and at m/z 121 and 149 for a methylenedioxypheyl ring. Matsuta et al. [62] demonstrated the detailed analysis of MS data obtained by GC–EI–MS for identification of cathinones and specified indexing information. However, the ionization rate of the fragments in ring-substituted cathinones is remarkably

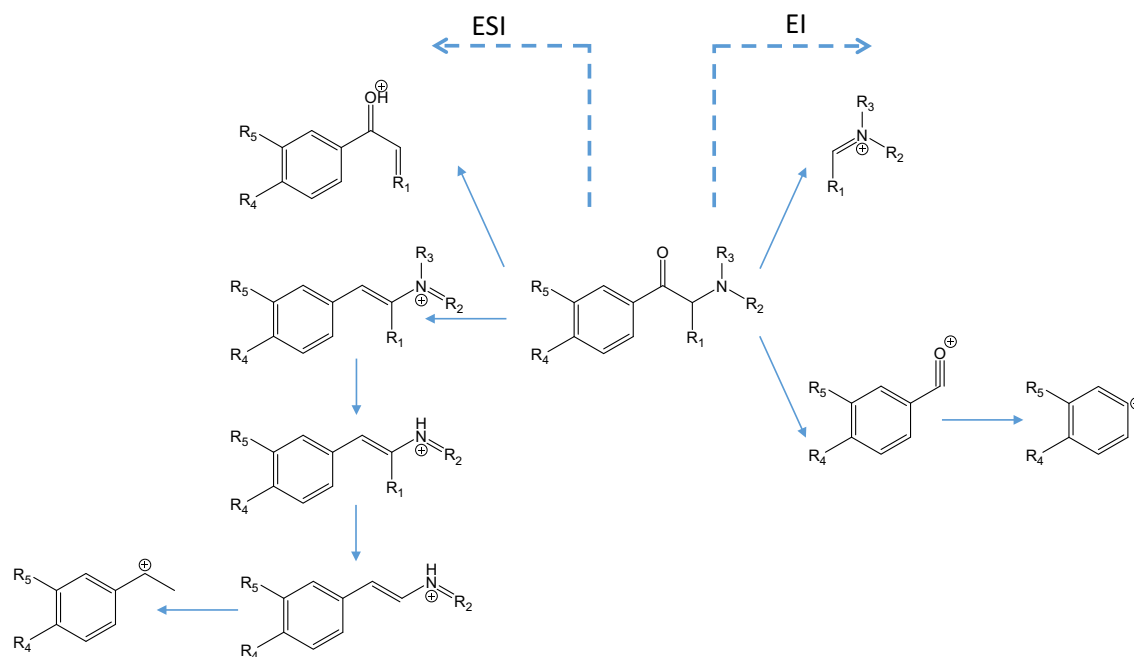


Fig. 5 Probable fragmentation pathways of cathinones by electrospray ionization and electron ionization (modified from references [61, 62, 65, 66])

weaker than that of the immonium ion. Other information obtained by TOFMS or CI-MS is helpful to delineate the molecular structure [63–66]. The identification of the regioisomer of the fluorinated cathinones was demonstrated using CI-MS [67]. However, this phenomenon was suggested to be limited to these analogs.

The published methods for analysis of cathinones in biological materials are summarized in Table 2 [68–79]. Simple LLE is usually used for extraction of cathinones from biological materials. The chromatographic conditions are also simple and do not usually require a special technique.

LC–MS–MS detection

The strategy for the detection of cathinones by LC–MS–MS is almost same as that for synthetic cannabinoids; almost all methods use MRM or SRM mode for sensitive determination. The probable fragmentation pathways are shown in Fig. 5. The $[M+H]^+$ ion is selected as a precursor ion, and three product ions that reflect the chemical structures of the cathinones are selected. Using this method, 30–50 drugs are monitored simultaneously in samples [80–82]. The published methods for analysis of cathinones in biological materials are summarized in Table 3 [68, 69, 74, 75, 77, 79, 82–98]. Simple LLE is usually used for the extraction of cathinones from biological materials. The chromatographic conditions are also simple and do not require a special technique.

In the same way as identification by GC–MS, other information obtained by TOFMS or tandem MS is needed to

clarify the molecular structure. An authentic drug or library database is needed to identify the drugs. Moreover, the probable fragmentation pathways of cathinones are described in the previous articles [61, 65, 66, 99]. These data are helpful in identifying the drugs.

As described in the section on synthetic cannabinoids, we occasionally encounter a dubious product that contains more than several milligrams of an unknown cathinone-like compound. In such a case, GC–MS, LC–MS, high-resolution MS (or TOFMS), and finally NMR spectroscopy are used to clarify the detailed chemical structure of the compound.

Concentrations in the cases of abuse

Synthetic cannabinoids

The common method of consumption of synthetic cannabinoids is smoking, which is the same as for conventional cannabis. The maximum concentrations of synthetic cannabinoids in serum are reached in less than 10 min after smoking [38]. The drugs absorbed in the body are metabolized smoothly, and the concentrations decrease rapidly. Moreover, there is also a report that cannabinoids accumulate in the adipose tissue because of their high lipophilicity [52]. Therefore, detection of the drug from serum is usually difficult. Synthetic cannabinoids absorbed in the human body are metabolized to hydroxyl or carboxyl derivatives of the aromatic ring or *N*-alkyl side chain [100]. It is difficult to identify the parent drug and its metabolites

Table 2 GC–MS conditions for cathinones in biological materials

Target(s)	Sample(s)	Purification	Derivatization(s)	Column(s)	LOD(s) (ng/ml)	Linear range (ng/ ml)	Reference
MDPV, metabolites	Urine	SPE (hydrolysis)	Methyl, acetyl, trimethylsilyl	HP-1 (12 m, 0.2 mm ID, 0.33 μ m) (Agilent)	–	–	[68]
MDPV, metabolites	Cellular fraction, urine	LLE (hydrolysis)	Trimethylsilyl	5 % Phenyl-methylsilicone (17 m, 0.2 mm ID, 0.33 μ m) (J and W)	2	10–2,000	[69]
Mephedrone, MDPV	Blood, urine	LLE		DB-1 (30 m, 0.32 mm ID, 0.25 μ m) (Agilent)	–	–	[70]
MDPV	Urine	LLE	Heptafluorobutyl	HP-5MS (12 m, 0.2 mm ID, 0.33 μ m) (Agilent), ZB-5MS (12 m, 0.2 mm ID, 0.33 μ m) (Phenomenex)	10	20–2,000	[71]
Methylone	Blood	LLE	Heptafluorobutyl	RTx-5 MS (30 m, 0.25 mm ID, 0.25 μ m) (Restek)	50	100–2,000	[72]
α -PVP, pyrovalerone (PV), MDPV	Blood	SPME		InertCap 5 (30 m, 0.25 mm ID, 0.25 μ m) (GL Sciences)	0.5 (PV, PVP), 1.0 (MDPV)	1–200	[73]
MDPV, α -PVP, α -PBP	Blood	LLE (Extrelut)		InertCap 5MS/NP (30 m, 0.25 mm ID, 0.25 μ m) (GL Sciences)	1	2–2,000	[74]
MDPV	Blood, tissue, urine	SPE		Zebtron Guardian ZB-50 (10 m, 0.18 mm ID, 0.18 μ m) (Phenomenex)	–	10–2,000	[75]
MDPV	Blood, urine	LLE		Rtx-5 ms (30 m, 0.25 mm ID, 0.25 μ m) (Restek)	–	–	[76]
3,4-Dimethylmethcathinone, metabolites	Urine	LLE	Trifluoroacetyl	DB-5MS (30 m, 0.25 mm ID, 0.25 μ m) (Agilent)	–	–	[77]
16 Synthetic cathinones	Urine	LLE	Trifluoroacetyl	CP7684 (10 m, 0.15 mm ID, 0.12 μ m) (Agilent)	–	–	[78]
α -PVP, metabolites	Urine	LLE	Trimethylsilyl	DB-5MS (30 m, 0.25 mm ID, 0.25 μ m) (Agilent)	–	–	[79]

Table 3 LC–MS or LC–MS–MS conditions for cathinones in biological materials

Target(s)	Sample(s)	Purification(s)	Column(s)	Mobile phase(s)	LOD(s) (ng/ml or g)	Linear range (ng/ml or g)	Reference
MDPV, metabolites	Urine	SPE (hydrolysis)	Hypersil Gold column (10 mm, 2.1 mm ID, 1.9 μ m) (Thermo Scientific)	10 mM ammonium formate (0.1 % formic acid), acetonitrile (0.1 % formic acid)			[68]
MDPV, metabolites	Cellular fraction, urine	LLE (hydrolysis)	Zorbax Eclipse Plus C18 (100 mm, 2.1 mm ID, 1.8 μ m) (Agilent)	0.1 % formic acid, acetonitrile (0.1 % formic acid)			[69]
MDPV	Serum	SPE	Phenyl–hexyl (50 mm, 3.0 mm ID, 3 μ m) (Phenomenex)	10 mM ammonium acetate (0.1 % formic acid), methanol	3	10–500	[83]
Mephedrone	Plasma	LLE	Synergi Fusion (150 mm, 4.6 mm ID)(Phenomenex); Spherisorb (150 mm, 4.6 mm ID) (Waters)	10 % acetonitrile (25 mM triethylammonium phosphate buffer), isocratic	39	78–10,000	[84]
9 Cathinones	Blood	PP	Prodigy Phenyl-3 (150 mm, 2.0 mm ID, 5 μ m) (Phenomenex)	0.1 % formic acid, methanol	0.5–3	10–400	[82]
7 Cathinones	Hair	LLE	Kintex PFP (50 mm, 2 mm ID, 2.6 μ m) (Phenomenex)	5 mM ammonium formate (pH 3.5), methanol (5 mM ammonium formate)	10–50 pg/mg		[85]
Butylone	Blood, liver	SPE	Allure PFP (50 mm, 2.1 mm ID, 5 μ m) (Restek)	0.02 % formic acid (2 mM of ammonium formate), acetonitrile	25 (blood)	50–2,000 (blood)	[86]
4-Methylethcathinone	Blood, urine	LLE	Zorbax SB-C18 (50 mm, 2.1 mm ID, 1.8 μ m) (Agilent)	0.1 % formic acid, acetonitrile (0.1 % formic acid)	0.96 (blood), 0.68 (urine)	10–1,000	[87]
MDPV, α -PVP, α -PBP	Hair	LLE (Extrelut)	Phenyl–hexyl (150 mm, 2.1 mm ID, 3 μ m) (Agilent)	10 mM ammonium formate (0.1 % formic acid, pH 3.3)/methanol (65:35), isocratic	0.02 ng/10-mm	0.05–50 ng/10-mm	[74]
Mephedrone	Blood	PP	Zorbax SB-C18 (50 mm, 2.1 mm ID, 1.8 μ m) (Agilent)	0.1 % formic acid, acetonitrile (0.1 % formic acid)	0.08	1–100	[88]
MDPV, mephedrone	Blood, plasma, urine	SPE	Kintex PFP (50 mm, 2.1 mm ID, 1.8 μ m) (Phenomenex)	2 mM ammonium formate (2 % formic acid), acetonitrile (0.1 % formic acid)	2	5–2,000	[89]
Mephedrone	Blood, urine	LLE	Zorbax SB-C18 (150 mm, 2.1 mm ID, 3.5 μ m) (Agilent)	0.1 % formic acid, methanol (0.1 % formic acid)	1 (blood), 2 (urine)	20–2,000	[90]

Table 3 continued

Target(s)	Sample(s)	Purification(s)	Column(s)	Mobile phase(s)	LOD(s) (ng/ml or g)	Linear range (ng/ml or g)	Reference
10 Cathinones	Blood, other specimens	LLE	Zorbax XDB-C18 (150 mm, 4.6 mm ID, 5 μ m) (Agilent)	5 mM ammonium acetate, methanol/acetonitrile	–	5–200	[91]
MDPV	Hair	SPE	Zorbax Eclipse Plus C18 (50 mm, 2.1 mm, 1.8 μ m) (Agilent)	0.1 % formic acid, acetonitrile	2.0 pg/mg	2–3,000 pg/ mg	[75]
MDPV	Blood	PP	Zorbax SB-C18 (50 mm, 2.1 mm ID, 1.8 μ m) (Agilent)	0.1 % formic acid, acetonitrile (0.1 % formic acid)	0.5	5–500	[92]
Buphedrone	Blood	PP	Zorbax SB-C18 (50 mm, 2.1 mm ID, 1.8 μ m) (Agilent)	0.1 % formic acid, acetonitrile (0.1 % formic acid)	0.3	1–1,000	[93]
3,4-Dimethylmethcathinone, metabolites	Urine	PP (hydrolysis)	L-column2 ODS (150 mm, 1.5 mm ID, 5 μ m) (Chemicals Evaluation and Research Institute)	10 mM ammonium formate buffer (pH 5), methanol	–	10–5,000	[77]
MDPV, metabolites	Plasma	PP (hydrolysis)	Synergy polar-RP (100 mm, 2 mm ID, 2.5 μ m) (Phenomenex)	0.1 % formic acid, acetonitrile (0.1 % formic acid)	0.1	0.25–1,000	[94]
α -PBP	Blood, urine, tissues	QuEChERS	Zorbax Eclipse Plus C18 (100 mm, 2.1 mm ID, 1.8 μ m) (Agilent)	10 mM ammonium formate (0.1 % formic acid), acetonitrile	0.05 (blood, urine), 0.1 (tissues)	8.6–4,280	[95]
3,4-Dimethylmethcathinone, metabolites	Blood, urine	QuEChERS	Shim-pack XR-ODS III (50 mm, 2.0 mm ID, 1.6 μ m) (Shimadzu); L-column2 ODS (150 mm, 1.5 mm ID, 5 μ m) (Chemical Evaluation and Research Institute)	10 mM ammonium formate, methanol; 10 mM ammonium formate (pH 5.0), methanol	1.03 (blood), 1.37 (urine)	5–400	[96]
MDPV metabolites	Urine	PP, LLE, SPE (hydrolysis)	Atlantis T3 (150 mm, 2.1 mm) (Waters)	10 mM ammonium formate buffer (0.1 % formic acid), acetonitrile (0.1 % formic acid)	–	–	[97]
α -PVP, metabolites	Urine	PP (hydrolysis)	L-column2 ODS (150 mm, 1.5 mm ID, 5 μ m) (Chemicals Evaluation and Research Institute)	10 mM ammonium formate (pH 5), methanol	–	10–10,000	[79]
PV9	Blood, urine	QuEChERS	Zorbax Eclipse Plus C18 (100 mm, 2.1 mm ID, 1.8 μ m) (Agilent)	10 mM ammonium formate (0.1 % formic acid), acetonitrile	0.05	10–1,000	[98]

in blood by GC–MS alone because the fragmentation of the metabolites is similar to that of the parent drug and analogs. Moreover, the concentration of the unchanged synthetic cannabinoids in blood is very low, and the number of metabolites that are commercially available is small. Low sensitivity is a limitation for the determination of synthetic cannabinoids in blood by GC–MS.

Although the concentration is influenced by the sampling time after drug intake and by the intake amount, concentrations of these drugs in serum were reported in the range of 0.1–190 ng/ml in poisoning cases [46]. In fatal cases, the concentrations of the drugs in blood were 0.1–199 ng/ml for JWH-018 and 0.1–68.3 ng/ml for JWH-073 [50], 12 ng/ml for AM-2201 [100], 1.1–1.5 ng/ml for 5F-PB-22 [53], and 12.4 ng/ml for MAM-2201 [52].

Cathinones

Unlike synthetic cannabinoids, the most common method of consumption of cathinones are insufflation (snorting) or ingestion. Inhalation, sublingual and rectal administration, and intramuscular or intravenous injection have also been reported. Unlike synthetic cannabinoids, the concentration of cathinones in blood is thought to vary because of the many modes of administration used by abusers. Only the blood concentration at one point and at several points have been quantified, and there is no report on continuous monitoring of the profile of the drug concentration in blood. The fatal concentration of the drug in blood was reported to be around 400 ng/ml [75]. The stability of cathinones in blood samples is clearly influenced by pH, as well as in the final extracts. In blood samples preserved with NaF/potassium oxalate, the measured concentrations of cathinone, methcathinone, ethcathinone, mephedrone, and flephedrone declined by ca. 30 % after 2 days of storage at 20 °C [82].

Some groups have studied the metabolic pathways of cathinones [68, 94, 101–103]. Unlike synthetic cannabinoids, the parent cathinones are detected easily in biological materials and are selected as the target because the unchanged parent drugs are rapidly excreted in urine. Cathinones are ionized in the body, and the reabsorption rate is low in the kidney because of low hydrophobicity. The excretion profile of α -PBP and α -PVP in human urine was determined after an intravenous injection, and the elimination half-life in urine was approximately 12 h. Moreover, the excreted amount in urine was influenced by urinary pH, like a psycho-stimulant [104]. To analyze these drugs in biological materials, it is necessary to remove endogenous substances from each sample and enrich the content of the drug. As shown in Table 3, LLE is usually used for extraction of the drugs from biological materials. The quantification of the metabolites is important to predict

the hazardous properties of the metabolites. However, because there are few metabolites marketed, no detailed study about their pharmacological activity or toxicity has been conducted.

In fatal cases, the concentrations of the drugs in blood were: 560–3,300 [72], 272 [105], and 60–1,120 ng/ml [106] for methylone; 1.2–22 [107], 5.1 [108], and 5.5 μ g/ml [88] for mephedrone; 55.2 ng/ml for α -PBP [95]; 486 [73] and 654 ng/ml [109] for α -PVP; 180 ng/ml [98] for PV9; 170 [70], 82 [110], 1,200 [74], 440 [75], 17–38 [92], and 700 ng/ml [111] for MDPV.

Conclusions

The number of abusers of synthetic cannabinoids and cathinones has increased remarkably worldwide. The chemical structures of the distributed drugs are skillfully changed so that the drugs may pass through screenings for detection. Simple screening methods are required for detection of these drugs in seized and biological materials. There are currently no commercial kits or devices for the routine screening of these drugs. Colorimetric, immunochemical, and chromatographic methods have been introduced in this review; a suitable method must be chosen for each laboratory. Although various human sample matrices are available for testing, urine and blood are of the first choices. However, many of these drugs, especially unchanged synthetic cannabinoids, exist in urine and blood for only a short period. Therefore, other matrices that can prove the consumption of these drugs, such as hair and saliva, are likely to receive more attention in the future.

Acknowledgments The authors are thankful to Professors Akira Ishii, Chief Editor, and Osamu Suzuki, Emeritus Chief Editor, Forensic Toxicology, for providing us an opportunity to write this review.

Conflict of Interest There are no financial or other relations that could lead to a conflict of interest.

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