

Analysis of 62 synthetic cannabinoids by gas chromatography–mass spectrometry with photoionization

Mamoru Akutsu¹  · Ken-ichi Sugie¹ · Koichi Saito²

Received: 13 July 2016 / Accepted: 24 September 2016 / Published online: 17 October 2016
© Japanese Association of Forensic Toxicology and Springer Japan 2016

Abstract Gas chromatography–mass spectrometry (GC–MS) in electron ionization (EI) mode is one of the most commonly used techniques for analysis of synthetic cannabinoids, because the GC–EI–MS spectra contain characteristic fragment ions for identification of a compound; however, the information on its molecular ions is frequently lacking. To obtain such molecular ion information, GC–MS in chemical ionization (CI) mode is frequently used. However, GC–CI–MS requires a relatively tedious process using reagent gas such as methane or isobutane. In this study, we show that GC–MS in photoionization (PI) mode provided molecular ions in all spectra of 62 synthetic cannabinoids, and 35 of the 62 compounds showed only the molecular radical cations. Except for the 35 compounds, the PI spectra showed very simple patterns with the molecular peak plus only a few fragment peak(s). An advantage is that the ion source for GC–PI–MS can easily be used for GC–EI–MS as well. Therefore, GC–EI/PI–MS will be a useful tool for the identification of synthetic cannabinoids contained in a dubious product. To the best of our knowledge, this is the first report to use GC–PI–MS for analysis of synthetic cannabinoids.

Keywords GC–MS · Photoionization · Synthetic cannabinoids · New psychoactive substances · Ionization chamber for electron ionization and photoionization in common

Introduction

In recent years, the harmful effects on human health from new psychoactive substances (NPSs), including synthetic cannabinoids, have become a serious social problem worldwide. Although almost all head shops selling the NPSs have been closed due to stringent control by the Ministry of Health, Labour and Welfare, as well as other organizations in Japan, such shops are still active in other countries. In addition, online sales activities continue at a rapid pace. The fastest and simplest method for identification of these NPSs is to record the electron ionization (EI) mass spectrum of a compound in question by gas chromatography–mass spectrometry (GC–MS) at 70 eV and to conduct a data search using the SWGDRUG Mass Spectral Library, for example, or Cayman Chemical Compounds Database. These databases are largely limited to EI mass spectra at 70 eV obtained by GC–MS, because of their excellent reproducibility. The only drawback of the EI mass spectra is that molecular or pseudo-molecular peaks are frequently very small or missing. For designer drugs including synthetic cannabinoids, there are many compounds showing very similar patterns of fragment peaks. In such cases, the appearance of a distinct molecular peak will be very useful for differentiating compounds with similar structures except for regioisomers.

The photoionization (PI) technique for MS is not new, having been developed about 60 years ago by Lossing and Tanaka [1]. After their discovery, PI received very little

✉ Mamoru Akutsu
akutsu-mamoru@mhlw.go.jp

¹ Narcotics Control Department, Kanto-Shin'etsu Regional Bureau of Health and Welfare, Ministry of Health, Labour and Welfare, 1-2-1 Kudan-Minami, Chiyoda-ku, Tokyo 102-8309, Japan

² Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41 Ebara 2-chome, Shinagawa-ku, Tokyo 142-8501, Japan

attention from the scientific community. However, in 2010, interest in PI was reignited with the description of the attachment of PI detection to “microfabricated planar glass GC”; the detection limit of mono-aromatics was sub-ng on columns [2]. A similar connection of PI to a portable GC system reported in 2013 showed a low detection limit less than 5 ppb for volatile organic compounds such as benzene, toluene, and styrene [3]. During our literature search on PI, we were surprised to find as many as seven articles on applications of PI published in international scientific journals in 2015–2016 [4–10]. Among these reports, various combinations of PI with atmospheric pressure MS [6, 7, 9] and with low-pressure MS [4, 10] were reported to provide extremely high sensitivity. It appears that PI technology is now under rapid development.

In the current study, we present GC–PI-MS spectra of as many as 62 kinds of synthetic cannabinoids, compared with those of GC–EI-MS spectra for the first time.

Materials and methods

Chemicals and their preparation

Sixty-two synthetic cannabinoids (for structures, see Fig. 1) were purchased from Cayman Chemical (Ann Arbor, MI, USA), and analytical-grade methanol and acetonitrile were obtained from Kanto Chemical (Tokyo, Japan). All but five synthetic cannabinoids were dissolved in methanol at a concentration of 100 µg/mL, to be used for mass spectra measurements; the exceptions were the five carboxylate compounds (FDU-PB-22, 5-fluoro-SDB-005, 5-fluoro-NPB-22, BB-22 and PB-22), each of which was dissolved in acetonitrile to give the same concentration in order to avoid thermal hydrolytic degradation in the presence of methanol [11].

GC–MS conditions

GC–MS analysis was conducted using an Agilent 7890B gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) connected to a JEOL JMS-Q1050 mass spectrometer with an EI/PI combination ion source (JEOL, Akishima, Japan) (Fig. 2). GC–PI-MS conditions were as follows: separation column, DB-5MS fused-silica capillary (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies); injector temperature, 230 °C; interface temperature, 150 °C; injection mode, splitless; injection volume, 2 µL; helium carrier gas flow rate, 1.0 mL/min; oven temperature program, initial temperature at 60 °C (1-min hold) followed by ramping at 10 °C/min to 150 °C (3-min hold) and then ramping at 10 °C/min to 300 °C (22-min hold); MS ionization mode, PI; wavelength range of

Fig. 1 Sixty-two synthetic cannabinoids classified into 13 types on the basis of structure. Compounds subjected to gas chromatography–mass spectrometry (GC–MS) in electron ionization (EI) mode (20 compounds in total) are shown by *double underlines*

vacuum ultraviolet (VUV) beam for PI by a deuterium lamp, 115–400 nm; transparent window between the VUV lamp and ion source, MgF₂; PI energy, 10.3 eV; ion source temperature, 150 °C; identification, scan mode; scan range, *m/z* 10–600. GC–EI-MS conditions were as follows: separation column, carrier gas flow rate, and oven temperature program, the same as those for GC–PI-MS; injector temperature, 250 °C; interface temperature, 200 °C; injection mode, split at 1:20; injection volume, 1 µL; electron energy, 70 eV; ion source temperature, 200 °C; identification, scan mode; scan range, *m/z* 40–500.

Results and discussion

Mass spectra obtained by GC–PI-MS

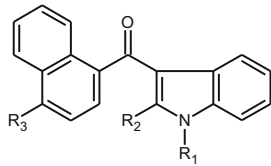
As an initial step in the GC–PI-MS methodology, it is very important to know the likelihood of molecular ion production for each target compound, depending on the ionization potential and the detector photon energy. In this work, we used 10.3 eV of photoionization energy that is used for general applications [12]. It is of great interest to obtain the mode of ionization for each synthetic cannabinoid. The 62 synthetic cannabinoids dealt with in this study encompassed almost every type of compounds. The GC–PI-MS protocol used here allowed us to observe the molecular ions for all 62 synthetic cannabinoids. The compounds used in this work can be categorized into three groups.

Group 1 comprises 35 compounds that generated only single molecular ions, including naphthoylindoles (19 compounds), carboxamide derivatives (5 compounds), benzoylindoles (5 compounds), naphthoylindazoles (2 compounds), naphthoylpyrroles (2 compounds), a naphthoylbenzimidazole (1 compound), and a naphthoylnaphthalene (1 compound) (Table 1).

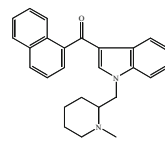
Group 2 compounds generated molecular ions as the base peak as well as smaller fragment ion(s): carboxamide derivatives (5 compounds), cyclopropyls (4 compounds), quinolinyl carboxylates (3 compounds), phenylacetylindoles (2 compounds), carboxyindoles (2 compounds), a naphthoyl carboxylate (1 compound), and a cyclohexylphenol (1 compound).

Group 3 compounds generated a small molecular ion and a fragment ion as a base peak: carboxamide derivatives (3 compounds), phenylacetylindoles (2 compounds), a naphthoylindole (1 compound), a benzoylindole (1

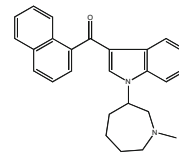
① Naphthoylindoles :



Generic scheduling of naphthoylindoles



AM1220



AM1220 azepan isomer

AM2232 : $R_1=C_5H_8N$, $R_2=H$, $R_3=H$

JWH-007 : $R_1=C_5H_{11}$, $R_2=CH_3$, $R_3=H$

JWH-016 : $R_1=C_4H_9$, $R_2=CH_3$, $R_3=H$

JWH-020 : $R_1=C_7H_{17}$, $R_2=H$, $R_3=H$

JWH-072 : $R_1=C_3H_7$, $R_2=H$, $R_3=H$

JWH-081 : $R_1=C_5H_{11}$, $R_2=H$, $R_3=OCH_3$

JWH-149 : $R_1=C_5H_{11}$, $R_2=CH_3$, $R_3=CH_3$

JWH-210 : $R_1=C_5H_{11}$, $R_2=H$, $R_3=C_2H_5$

JWH-398 : $R_1=C_5H_{11}$, $R_2=H$, $R_3=Cl$

EAM-2201 : $R_1=C_5H_{10}F$, $R_2=H$, $R_3=H$

JWH-015 : $R_1=C_3H_7$, $R_2=CH_3$, $R_3=H$

JWH-019 : $R_1=C_6H_{13}$, $R_2=H$, $R_3=H$

JWH-022 : $R_1=C_4H_8$, $R_2=H$, $R_3=H$

JWH-080 : $R_1=C_4H_9$, $R_2=H$, $R_3=OCH_3$

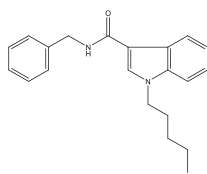
JWH-098 : $R_1=C_5H_{11}$, $R_2=CH_3$, $R_3=OCH_3$

JWH-182 : $R_1=C_5H_{11}$, $R_2=H$, $R_3=C_3H_7$

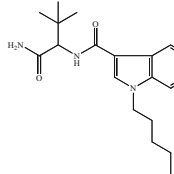
JWH-213 : $R_1=C_5H_{11}$, $R_2=CH_3$, $R_3=C_2H_5$

JWH-412 : $R_1=C_5H_{11}$, $R_2=H$, $R_3=F$

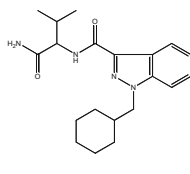
② Carboxamide derivatives :



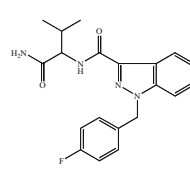
SDB-006



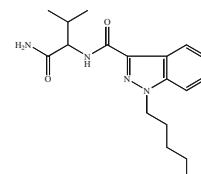
ADBICA



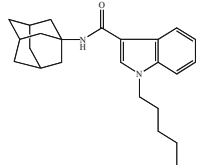
AB-CHMINACA



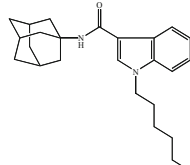
AB-FUBINACA



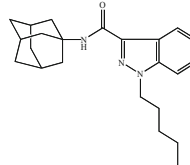
AB-PINACA



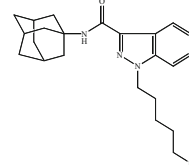
APICA



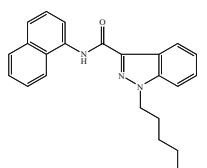
APICA
N-(5-fluoropentyl)
derivative



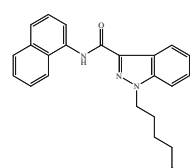
APINACA
N-(5-fluoropentyl)
derivative



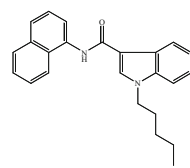
APINACA



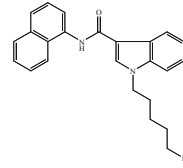
MN-18



5-Fluoro-MN-18

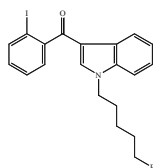


NNE1

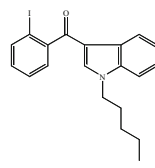


5-Fluoro-NNE1

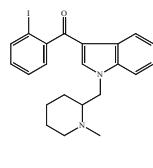
③ Benzoylindoles :



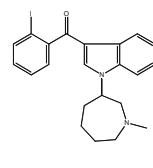
AM694



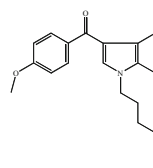
AM679



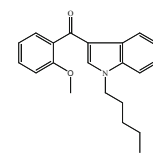
AM2233



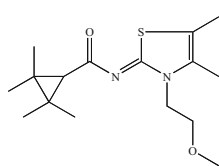
AM2233
azepan isomer



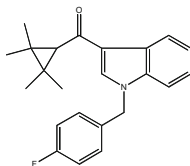
RCS-4



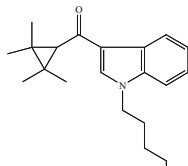
RCS-4
ortho isomer

④Cyclopropyls :

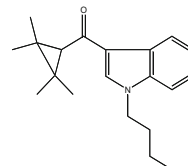
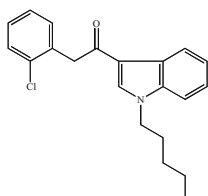
A-836339



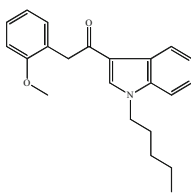
FUB-144



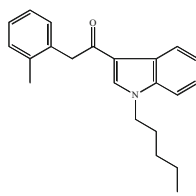
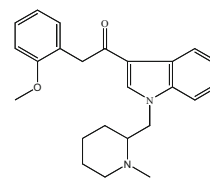
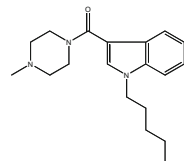
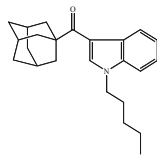
UR-144

XLR-12**⑤Phenylacetylindoles :**

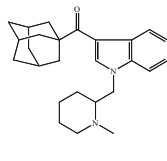
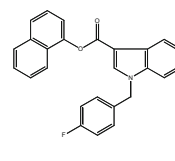
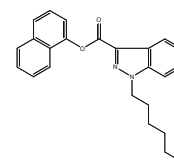
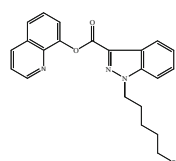
JWH-203



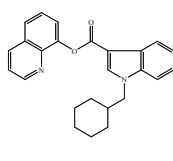
JWH-250

JWH-251Cannabipiperidiethanone**⑥Carboxyindoles :**MEPIRAPIM

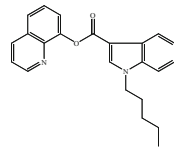
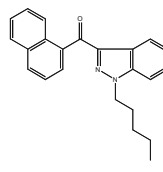
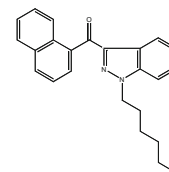
AB-001

AM1248**⑧Naphthoyl carboxylates :**FDU-PB-225-Fluoro-SDB-005**⑦Quinolinyln carboxylates :**

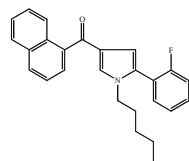
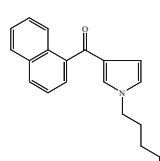
5-Fluoro-NPB-22



BB-22

PB-22**⑨Naphthoylindazoles :**THJ-018

THJ-2201

⑩Naphthoylpyrroles :JWH-307

JWH-030

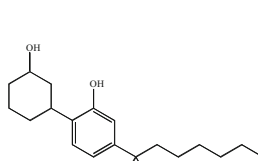
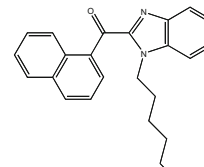
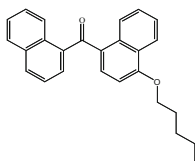
⑪Cyclohexylphenol :CP-47,497**⑫Naphthoylbenzimidazole :**FUBIMINA**⑬Naphthoynaphthalene :**CB-13

Fig. 1 continued

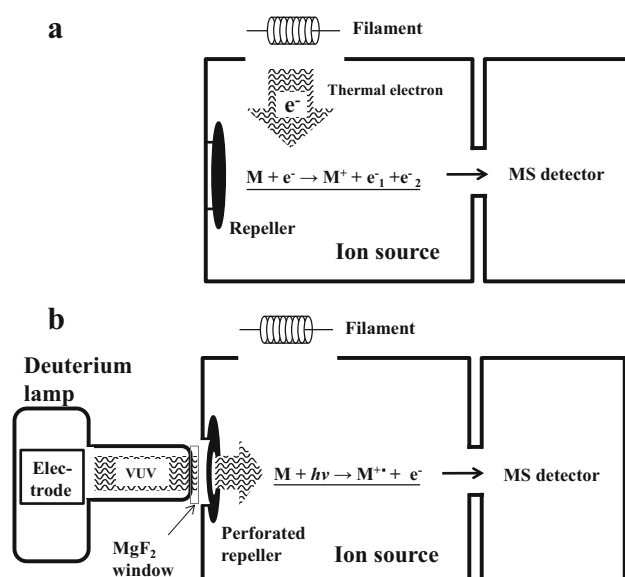


Fig. 2 Schematic illustration of the EI and photoionization (PI)/EI combination source. **a** EI ion source: irradiation by thermal electrons generated from the filament to the sample. **b** PI/EI combination source: irradiation by vacuum ultraviolet (VUV) light from the ultraviolet lamp to the sample. It was possible to continuously obtain fragment ion information by EI and molecular ion information by PI

compound), a carboxyindole (1 compound), and a naphthoyl carboxylate (1 compound) (Table 1).

The PI technique is unique in that the radical cation is produced by ultraviolet light radiation by depriving one electron from a target molecule with a low ionization threshold [6], while EI requires the deprivation of two electrons at a time for ion formation with relatively high ionization threshold. Therefore, the PI technique has been used as a convenient method for detecting stable neutral compounds such as volatile organic compounds or neutral oil components [2, 3, 6, 7, 9].

In the present study, which dealt with 62 synthetic cannabinoids, all compounds were able to be detected by GC–PI–MS. Furthermore, as many as 35 compounds showed only the molecular ions in their mass spectra, without the appearance of any fragment peaks, as described above. The compounds with fewer functional groups tended to show single molecular peaks in their mass spectra, such as the group of naphthoylindoles (19 compounds) (Table 1).

Figure 3 shows examples of mass spectra in the PI and EI modes for eight selected synthetic cannabinoids. While there were various types of mass spectra observed in the PI mode, all spectra showed peaks of molecular ions. In contrast, in the EI mode, the molecular/quasi-molecular peaks were not detected in two of the eight spectra (Fig. 3b,

g). When fragment peak(s) appeared in a PI spectrum, the same fragment peak(s) also appeared in the corresponding EI spectrum (Fig. 3b, c, g).

In the EI mode, 70 eV of ionization energy is typically used, because the high energy provides the most stable and reproducible results. Such a phenomenon is also the case for the PI mode; ionization energy of 10.0–10.6 eV is typically used for general applications [1, 12]. In the present study, 70 eV EI and 10.3 eV PI were used. Except for the ease in producing the molecular radical cations, the fragmentation modes in the EI and PI modes seem essentially similar, as exemplified with AB-CHMINACA, XLR-12, and AM1220 (Fig. 3b, c, g). As shown in the PI mode (Table 1), many synthetic cannabinoids showed a molecular radical cation together with a fragment ion (Fig. 3b, d, e–g), but only a few PI spectra showed more than one fragment peak (Fig. 3c). This means that most of the free electrons that played a role in creating a fragment ion became extinct at this stage. In the EI mode, the multiple free electrons generated have no capability of producing a molecular radical cation, but act to produce various types of fragment ions via the cleavage of bonds in α - and/or β -position against a heteroatom. It seems correct that fragmentation is nearly the same between the PI and EI modes at the early stage (Fig. 4), except for the unique capacity of the PI to produce molecular radical cations with a low threshold.

Here, we must mention GC–MS in chemical ionization (CI) mode. This technique is used to estimate the molecular weight of a target compound, and actually gives an intense peak due to $[M + H]^+$, but sometimes gives an adduct ion produced by molecular binding with a reagent gas or its fragment, which makes the interpretation complicated. In addition, GC–CI–MS requires a reagent gas such as methane or isobutane, various conditions of which should be optimized beforehand; such a procedure is tedious (e.g., reagent gas selection, flow rate adjustment). Although it is possible for GC–CI–MS to share the same ionization chamber with GC–EI–MS, it is now common to use different GC–EI–MS and GC–CI–MS systems in order to use them under their optimal analytical conditions. In this respect, the PI device can be attached to most GC–EI–MS instruments under optimal conditions to share the same ionization chamber for both EI and PI modes. In our opinion, attaching the PI system to the EI chamber is the best option, because of the absence of adduct formation and low cost of installation. The PI technique appears to be superior to the CI mode for obtaining information on the molecular weight of a target compound.

Table 1 Grouping of the 13 types of synthetic cannabinoids according to mass spectra by gas chromatography–mass spectrometry in photoionization mode

Group 1 Molecular ion only (35 compounds)

① Naphthoylindoles (19 compounds)

AM2232, EAM-2201, JWH-007, JWH-015, JWH-016, JWH-019, JWH-020, JWH-022, JWH-072, JWH-080, JWH-081, JWH-098, JWH-149, JWH-182, JWH-210, JWH-213, JWH-398, JWH-412, AM1220 azeapan isomer

② Carboxamide derivatives (5 compounds)

SDB-006, APICA, APICA JV-(5-fluoropentyl) derivative, MN-18, 5-fluoro-MN-18

③ Benzoylindoles (5 compounds)

AM679, AM694, RCS-4, RCS-4 ortho isomer, AM2233 azeapan isomer

⑨ Naphthoylindazoles (2 compounds)

THJ-018, THJ-2201

⑩ Naphthoylpyrroles (2 compounds)

JWH-307, JWH-030

⑫ Naphthoylbenzimidazole (1 compound)

FUBIMINA

⑬ Naphthoylnaphthalene (1 compound)

CB-13

Group 2 Molecular ion with smaller fragment ion(s) (18 compounds)

② Carboxamide derivatives (5 compounds)

APINACA, APINACA JV-(5-fluoropentyl) derivative, NNE1, 5-fluoro-NNE1, ADBICA

④ Cyclopropyls (4 compounds)

A-836339, FUB-144, UR-144, XLR-12

⑦ Quinoliny carboxylates (3 compounds)

BB-22, PB-22, 5-fluoro-NPB-22

⑤ Phenylacetylindoles (2 compounds)

JWH-250, JWH-251

⑥ Carboxyindoles (2 compounds)

MEPIRAPIM, AB-001

⑧ Naphthoyl carboxylate (1 compound)

5-Fluoro-SDB-005

⑪ Cyclohexylphenol (1 compound)

CP-47,497

Group 3 Smaller molecular ions with a fragment ion as base peak (9 compounds)

② Carboxamide derivatives (3 compounds)

AB-CHMINACA, AB-FUBINACA, AB-PINACA

⑤ Phenylacetylindoles (2 compounds)

JWH-203, cannabipiperidiethanone

① Naphthoylindole (1 compound)

AM1220

③ Benzoylindole (1 compound)

AM2233

⑥ Carboxyindole (1 compound)

AM1248

⑧ Naphthoyl carboxylate (1 compound)

FDU-PB-22

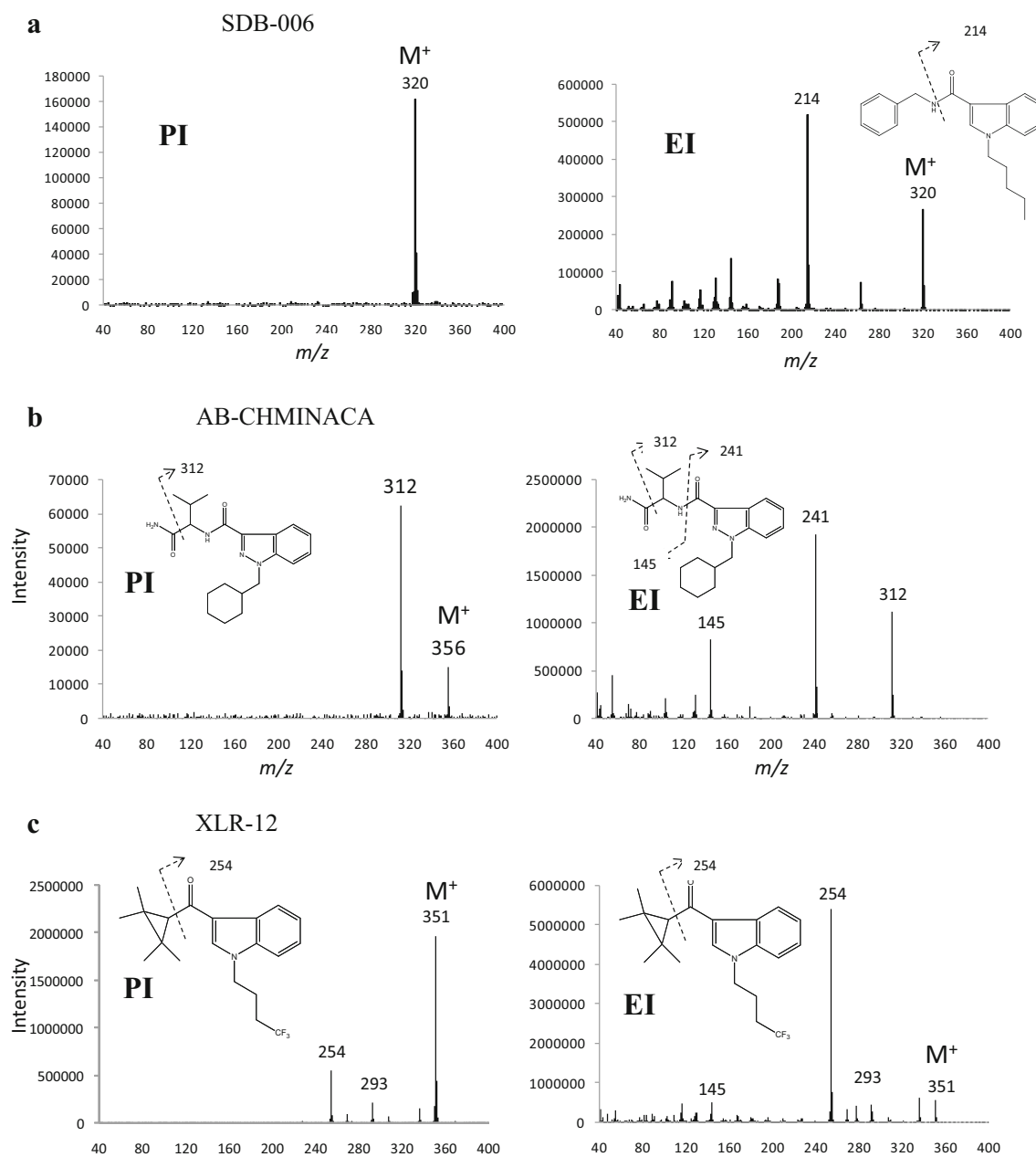


Fig. 3 Examples of mass spectra for synthetic cannabinoids obtained by GC–MS in PI and EI modes and their probable fragmentation pathways

Conclusions

In this study, the mass spectra of 62 synthetic cannabinoids were recorded by GC–PI-MS, and the mass spectra for 20 compounds were measured by GC–EI-MS for comparison. Most of the mass spectra obtained from the 62 synthetic cannabinoids by GC–PI-MS showed very simple patterns of either molecular ion only or a molecular ion plus only

one fragment peak, providing useful information on the molecular weight of the target compound. Therefore, GC–EI/PI-MS will be a useful tool for identifying synthetic cannabinoids contained in a dubious product. The information on the ease of producing molecular radical cations by GC–PI-MS for synthetic cannabinoids will aid in the construction of a highly sensitive MS method using PI technology for analysis of a specific synthetic cannabinoid

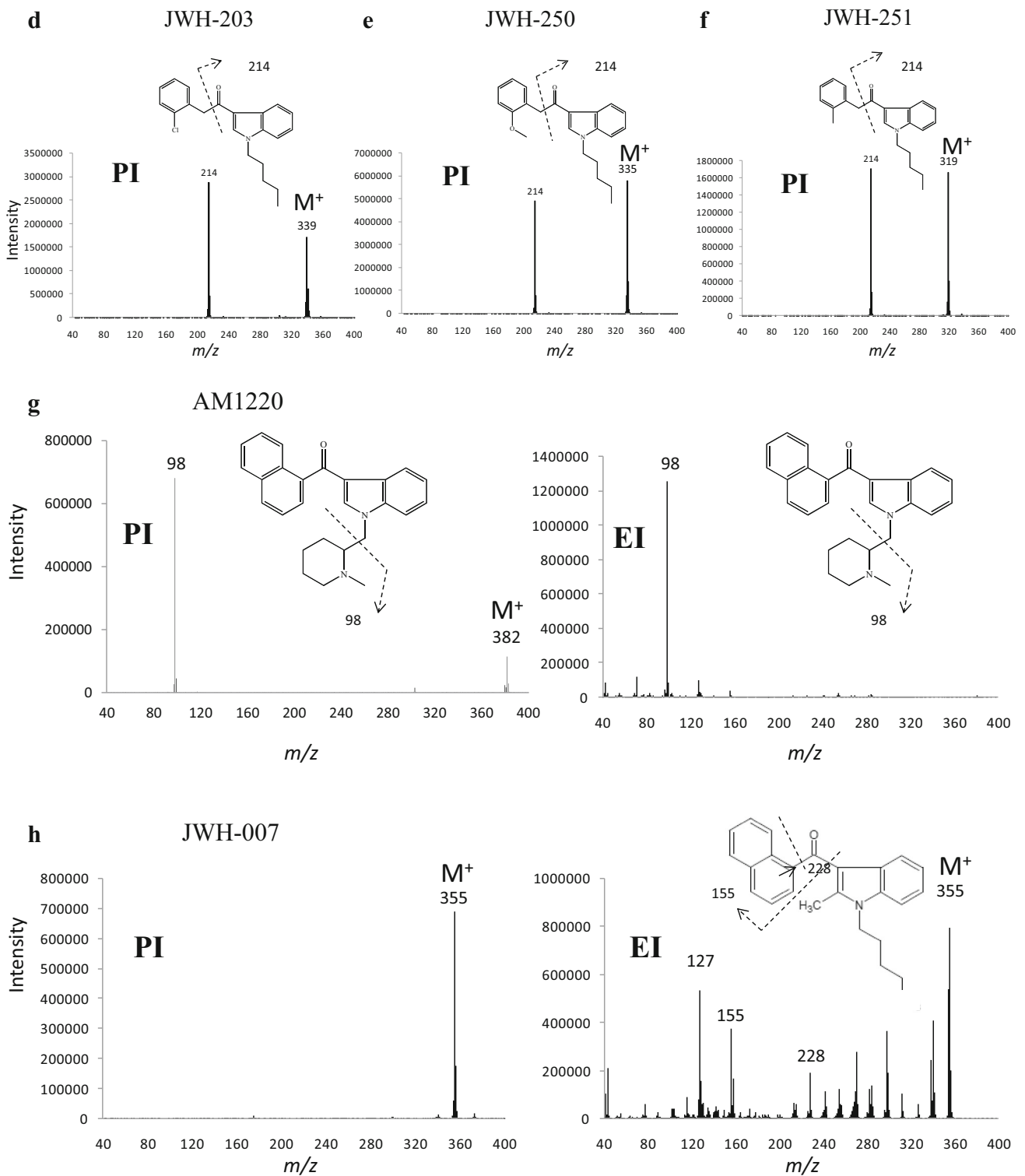
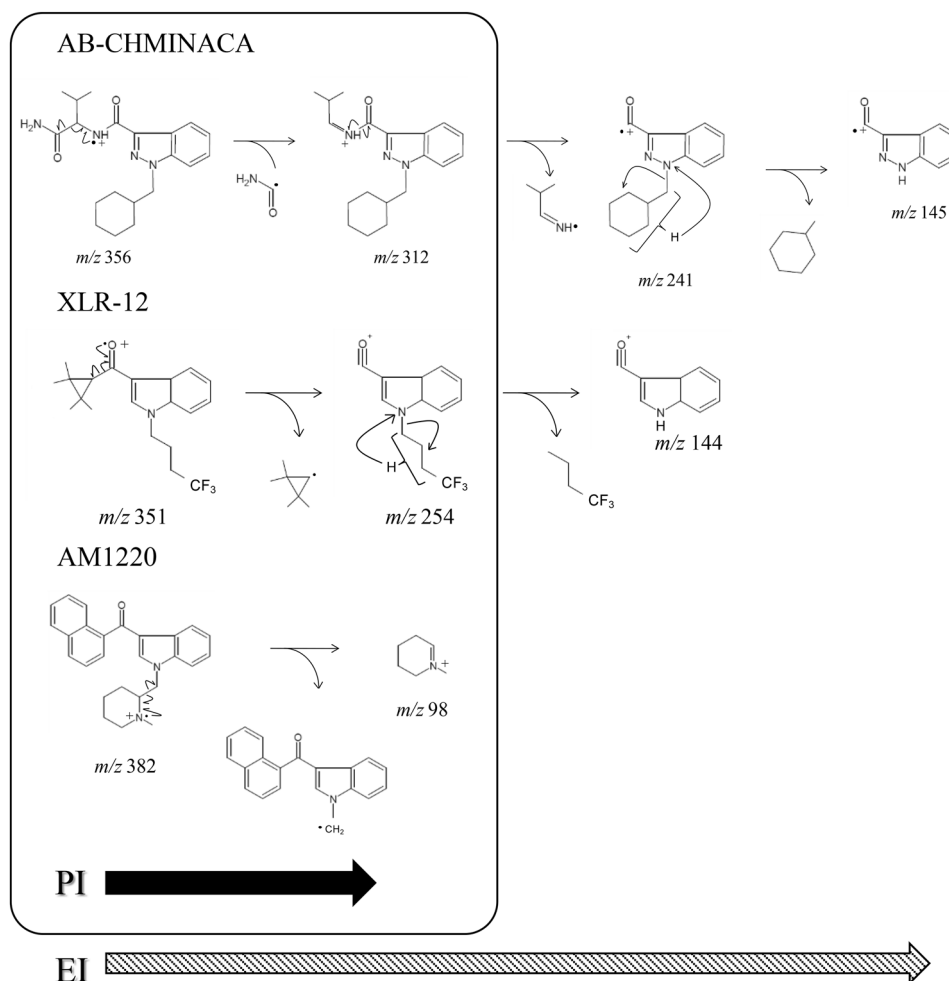


Fig. 3 continued

Fig. 4 Proposed mechanism of cleavage to form fragment ions in three synthetic cannabinoids measured by PI and EI. Further fragmentation occurred after cleavage of the α -bond in AB-CHMINACA and XLR-12 subjected to GC–EI-MS. No further fragmentation occurred in these compounds by GC–PI-MS



in biological samples. To our knowledge, this is the first report to use the PI technology for analysis of synthetic cannabinoids.

Acknowledgments A portion of this work was supported by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan.

Compliance with ethical standards

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Lossing FP, Tanaka K (1956) Photoionization as a source of ions for mass spectrometry. *J Chem Phys* 25:1031–1034
- Lewis AC, Hamilton JF, Rhodes CN, Halliday J, Bartle KD, Homewood P, Grenfell RJP, Goody B, Harling AM, Brewer P, Vargha G, Milton MJT (2010) Microfabricated planar glass gas chromatography with photoionization detection. *J Chromatogr A* 1217:768–774
- Sun J, Guan F, Cui D, Chen X, Zhang L, Chen J (2013) An improved photoionization detector with a micro gas chromatography column for portable rapid gas chromatography system. *Sensor Actuat B-Chem* 188:513–518
- Liu C, Zhu Y, Zhou Z, Yang J, Qi F (2015) Ultrasonic nebulization extraction/low pressure photoionization mass spectrometry for direct analysis of chemicals in matrices. *Anal Chim Acta* 891:203–210
- Sun W, Zhang P, Yang B, Shu J, Wang Y, Li Y (2015) Products and mechanisms of the heterogeneous reaction of three suspended herbicide particles with NO_3 radicals. *Sci Total Environ* 514:185–191
- Revelsky IA, Tikhonova IN, Yashin YS (2015) Fast detection of polycyclic aromatic hydrocarbons in complex mixtures of organic compounds based on gas chromatography–mass spectrometry with atmospheric pressure photoionization. *Eur J Mass Spectrom* 21:753–757
- Kersten H, Kroll K, Haberer K, Brockmann KJ, Benter T, Peterson A, Makarov A (2016) Design study of an atmospheric pressure photoionization interface for GC–MS. *J Am Soc Mass Spectrom* 27:607–614
- Zacs D, Bartkevicz V (2015) Analytical capabilities of high performance liquid chromatography–atmospheric pressure photoionization–Orbitrap mass spectrometry (HPLC–APPI–

- Orbitrap–MS) for trace determination of novel and emerging flame retardants in fish. *Anal Chim Acta* 898:60–72
9. Lung S-CC, Liu C-H (2015) Fast analysis of 29 polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs with ultra-high performance liquid chromatography–atmospheric pressure photoionization–tandem mass spectrometry. *Sci Rep* 5:12992. doi:[10.1038/srep12992](https://doi.org/10.1038/srep12992)
 10. Sun W, Liang M, Li Z, Shu J, Yang B, Zou Y (2016) Ultrasensitive detection of explosives and chemical warfare agents by low-pressure photoionization mass spectrometry. *Talanta* 156–157:191–195
 11. Tsujikawa K, Yamamuro T, Kuwayama K, Kanamori T, Iwata YT, Inoue H (2014) Thermal degradation of a new synthetic cannabinoid QUPIC during analysis by gas chromatography–mass spectrometry. *Forensic Toxicol* 32:201–207
 12. Poole CF (2015) Ionization-based detectors for gas chromatography. *J Chromatogr A* 1421:137–153