Research article

Journal of MASS SPECTROMETRY

Received: 17 August 2015 Revised: 20 October 2015 Accepted: 20 October 2015 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jms.3722

Fragmentation of synthetic cannabinoids with an isopropyl group or a *tert*-butyl group ionized by electron impact and electrospray

Shigeki Akamatsu* and Masashi Yoshida

This study described a fragmentation pattern of 21 synthetic cannabinoids with an isopropyl group or a *tert*-butyl group by electron impact ionization quadrupole mass spectrometry and electrospray ionization time-of-flight mass spectrometry in the positive mode. The compounds were categorized into four types according to substituted group such as a terminal amide and ester. The characteristic fragment ion in each group was obtained. The main common fragment ions for the two ionizations were formed by C–N cleavage of the amide group adjacent to the *N*-hetero rings. Additionally, the fragment ions indicated the difference in the basic structure as well as substituted group, which are useful for estimating the chemical structures of unknown compounds.

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Keywords: fragmentation pattern; mass spectrometry; synthetic cannabinoid; isopropyl group; *tert*-butyl group

Introduction

Synthetic cannabinoids, one of new psychoactive substances, have become disseminated worldwide since 2009 by the international diffusion of the so-called 'Spice' products [1]. The synthetic cannabinoids have been reported to have a high affinity for cannabinoid receptors. Many cannabimimetic compounds were controlled as designated substances in Japan. However, new designer compounds have been created by means of slight structural changes of known illicit compounds. Cannabimimetic derivatives with an isopropyl group or a *tert*-butyl group, such as AB-FUBINACA, ADB-FUBINACA, and ADBICA, have been widely distributed since 2012 and controlled subsequently. Twenty-two compounds with the isopropyl or the *tert*-butyl group are controlled as of July 2015 in Japan, and derivatives with their groups will have been increasing in the future.

Analytical methods for the identification and quantification of synthetic cannabinoids need to be reliable and accurate for routine monitoring and criminal forensics. Several analytical approaches are generally used including the following: high-performance liquid chromatography/photo-diode array detection, liquid chromatography–mass spectrometry (LC/MS), and gas chromatography/mass spectrometry (GC/MS) [2–5]. Mass and absorption spectra obtained by these techniques are compared with the reference standard to identify the detected compound. Without the reference standard, the compound must be estimated from a detailed study of the mass spectra. The accurate estimation of chemical structure is the fastest way to identify the unknown compound.

Mass spectral fragmentation analyses in small molecule compound including psychoactive substances have been reported so far [6–8]. Most studies have shown characteristic fragmentation based on the electron impact MS (EI-MS) using GC/MS. However, to our knowledge, few studies for psychoactive substances have attempted to the electrospray ionization MS (ESI-MS) using LC/MS. In this study, the detailed fragmentation analysis of both EI-MS and ESI-MS spectra in synthetic cannabinoids with the isopropyl group or the *tert*-butyl group using 21 available reference standards was conducted. The result was that we summarized the fragmentation pattern of these cannabinoids.

Materials and methods

Chemicals

Twenty-one synthetic cannabinoid standards (Table 1) were purchased from Cayman Chemical (Ann Arbor, MI). Ammonium acetate and methanol were purchased from Wako Pure Chemical (Osaka, Japan). Acetonitrile was purchased from Sigma-Aldrich (St. Louis, MO). Methanol and acetonitrile were of LC/MS grade. Water was purified by a Milli-Q Water Purification System (Millipore, Billerica, MA). The stock standard solutions (250 mg/ml each) were prepared in methanol and were stored at −20 °C. The working standard solutions were also prepared by mixing the stock solutions and diluting with methanol just prior to measurement. The concentrations of the working solutions for GC/MS analysis and LC/MS analysis were 10 and 0.1 mg/ml, respectively.

Instrumentation

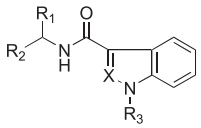
The analyses were carried out on an Agilent 6890N GC apparatus (Palo Alto, CA) coupled with an Agilent 5973 inert quadrupole mass spectrometer and an Agilent 1200 LC with an Agilent 6210 time of flight mass spectrometer.

* *Correspondence to: Shigeki Akamatsu, Hyogo Prefectural Institute of Public Health and Consumer Sciences, 2-1-29, Arata-cho, Hyogo-ku, Kobe 652-0032, Japan. E-mail: shigeki_akamatsu@pref.hyogo.lg.jp*

Hyogo Prefectural Institute of Public Health and Consumer Sciences, 2-1-29, Arata-cho, Hyogo-ku, Kobe 652-0032, Japan

Journal of MASS SPECTROMETRY

Table 1. Chemical structures of the analytes



No.	Name	X	R_1	R_2	R_3
1	ADBICA	CH (indole)	$C(CH_3)_3$ (*tert*-butyl)	$CO–NH_2$	pentyl
2	5F-ADBICA	CH	$C(CH_3)_3$	$CO–NH_2$	5-fluoropentyl
3	MDMB-CHMICA	CH	$C(CH_3)_3$	$CO–OCH_3$	cyclohexylmethyl
4	5F-ABICA	CH	$CH(CH_3)_2$ (isopropyl)	$CO–NH_2$	5-fluoropentyl
5	MMB2201	CH	$CH(CH_3)_2$	$CO–OCH_3$	5-fluoropentyl
6	ADB-PINACA	N (indazole)	$C(CH_3)_3$	$CO–NH_2$	pentyl
7	5F-ADB-PINACA	N	$C(CH_3)_3$	$CO–NH_2$	5-fluoropentyl
8	ADB-CHMINACA	N	$C(CH_3)_3$	$CO–NH_2$	cyclohexylmethyl
9	ADB-FUBINACA	N	$C(CH_3)_3$	$CO–NH_2$	4-fluorobenzyl
10	5F-ADB	N	$C(CH_3)_3$	$CO–OCH_3$	5-fluoropentyl
11	MDMB-CHMINACA	N	$C(CH_3)_3$	$CO–OCH_3$	cyclohexylmethyl
12	MDMB-FUBINACA	N	$C(CH_3)_3$	$CO–OCH_3$	4-fluorobenzyl
13	AB-PINACA	N	$CH(CH_3)_2$	$CO–NH_2$	pentyl
14	5F-AB-PINACA	N	$CH(CH_3)_2$	$CO–NH_2$	5-fluoropentyl
15	5Cl-AB-PINACA	N	$CH(CH_3)_2$	$CO–NH_2$	5-chloropentyl
16	AB-CHMINACA	N	$CH(CH_3)_2$	$CO-NH_2$	cyclohexylmethyl
17	AB-FUBINACA	N	$CH(CH_3)_2$	$CO-NH_2$	4-fluorobenzyl
18	AMB	N	$CH(CH_3)_2$	$CO–OCH_3$	pentyl
19	5F-AMB	N	$CH(CH_3)_2$	$CO–OCH_3$	5-fluoropentyl
20	MA-CHMINACA	N	$CH(CH_3)_2$	$CO–OCH_3$	cyclohexylmethyl
21	FUB-AMB	N	$CH(CH_3)_2$	$CO–OCH_3$	4-fluorobenzyl

The GC separation was performed on an HP-1MS column (Agilent, 30 m, 0.25 mm i.d., and 0.25 μm) with a Siltek guard column (Restek, Bellefonte, PA, 1 m, 0.25 mm i.d.). The column temperature program was set as follows: 200 °C hold for 1 min, increase at 5 °C/min to 310 °C, and hold for 17 min. The injector was held at 250 °C, and the carrier gas was helium at a flow rate of 1.1 ml/min. The injection volume was 1 μl in splitless mode. The mass spectrometer was operated in electron impact ionization mode with an electron energy of 70 eV. The other parameters were as follows: full scan mode, *m/z* 50–700; ion source temperature, 230 °C; and quadrupole temperature, 150 °C.

The LC column was a Poroshell 120 EC-18 (Agilent, 3.0 × 50 mm, 2.7 μm), and the guard column was a Inertsil ODS-3 (GL Science, Tokyo, Japan, 3.0 × 10 mm, 3 μm). The mobile phase consisted of water with 10 mM ammonium acetate (A) and acetonitrile (B) at a flow rate of 0.5 ml/min, with the gradient elution program as follows: 0 min, 10% B; 3 min, 50% B; and 10 min 95% B (5 min hold). The column temperature was 40 °C, and the injection volume was 1 μl. The mass spectrometric analysis was performed using the following parameters: ionization mode, ESI positive; capillary voltage, 4000 V; fragmentor voltage, 100, 200, 300, and 350 V; drying gas (N_2) flow rate, 10 l/min; drying gas temperature, 350 °C; scan range, *m/z* 50–1000; and reference masses, 121.0509 and 922.0098.

Results and discussion

EI-MS spectra

Representative EI-MS spectra of synthetic cannabinoids with an isopropyl group and a *tert*-butyl group are shown in Fig. 1. All remaining EI-MS spectra are shown in Fig. S1. The molecular ions were observed with very low abundance as expected, whereas the high-abundance fragment ions showed the characteristics of the chemical structures.

The major fragment ions of analytes were formed by C–N cleavage of the amide group adjacent to the *N*-hetero rings. The fragment ions revealed the structures of the *N*-hetero ring side. When the *m/z* value of this fragment ion was an odd number, the compound had an indazole ring. Meanwhile, when the value was an even number, the compound had an indole ring. Furthermore, the value suggested the difference of a moiety attached to the *N* atom of *N*-hetero ring (R_3 group in Table 1). The fragment ions formed by desorption of *N*-hetero ring from those ions also indicated the presence of the R_3 group. Just as an example, the fragment ions at *m/z* 109, 145, and 253 suggested the presence of a fluorobenzyl moiety ($–CH_2–C_6H_4F$, *m/z* 109) as the R_3 group and an indazole ring because the ion formed by C–N cleavage of the amide group (*m/z* 253) was an odd number. The ion at *m/z* 145 represents the fragment ion formed by desorption of the R_3 group from the ion at *m/z* 253.

The fragment ion generated by the desorption of the terminal halogen atom such as fluorine and chlorine was also useful for identifying the R_3 group. This fragmentation occurred exclusively in alkyl side chain, although the halogen atom in aromatic ring such as FUB-AMB was not dissociated. The ion at *m/z* 213 was formed by the halogen elimination of the ion at *m/z* 233 in 5F-AMB [Fig. 1(A)].

It is worth noting that the abundances of the amide cleavage fragment ions bound to the R_3 group were higher than those of the amide cleavage ions formed by the loss of the R_3 group. For example, the ion at *m/z* 214 was higher than the ion at *m/z* 144 in

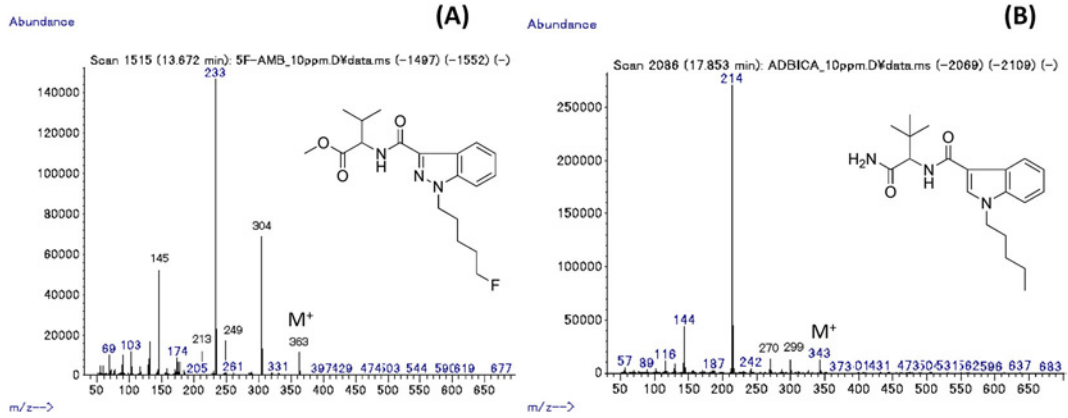


Figure 1. EI-MS spectra of 5F-AMB (A) and ADBICA (B).

ADBICA [Fig. 1(B)]. This means that the C–N cleavage of the amide group is given priority over the loss of the R_3 group.

The fragment patterns differed between compounds with the isopropyl group and the *tert*-butyl group (R_1 group in Table 1). Additionally, the fragmentation was affected by the terminal substituted group (R_2 group in Table 1). The compounds were categorized into four types in terms of R_1 group and R_2 group. Fig. 2 shows the proposed fragmentation mechanisms for four types of compounds. In compounds with the isopropyl group, a C–N bond between the isopropyl group and the amide group was dissociated regardless of the R_2 group. The ion at *m/z* 265 and 269 was generated by the C–N cleavage in 5Cl-AB-PINACA [Fig. 2(A)] and FUB-AMB [Fig. 2(C)], respectively. This mechanism is designated as McLafferty rearrangement, associated with γ-H shift and β-cleavage (Fig. 3)[9]. The γ-H is essential for this C–N cleavage, suggesting that this fragment ion is unique to the compounds with the isopropyl group. The C–N cleavage was not actually detected in compounds with the *tert*-butyl group, because there is no γ-H.

When the R_1 group was the *tert*-butyl, the fragment patterns varied with the R_2 group. The fragment ions formed by loss of terminal amide (R_2 = CO–NH_2, *m/z* 44) from the molecular ions were detected for all compounds with the terminal amide (α-cleavage of amide group) [Fig. 2(B)]. The abundance of $[M-44]^+$ was relatively high, which indicates that the desorption of the terminal amide occurs first. The

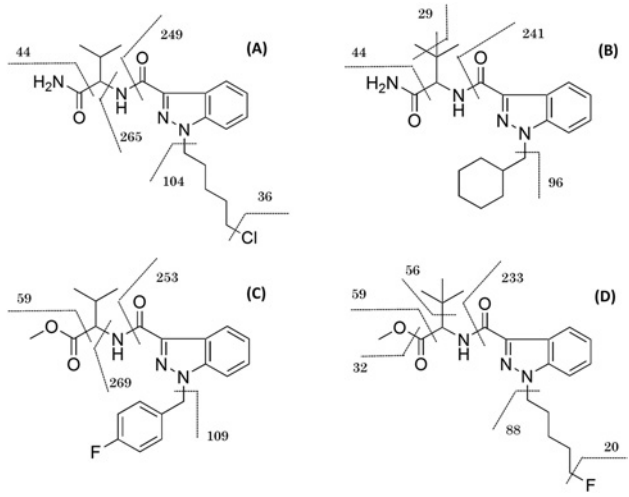


Figure 2. Proposed mass spectral fragmentation for 5Cl-AB-PINACA (A), ADB-CHMINACA (B), FUB-AMB (C), and 5F-ADB (D).

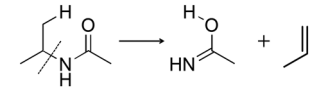


Figure 3. Proposed mechanism of McLafferty rearrangement.

fragment ion of $[M-73]^+$ formed by loss of *m/z* 29 from that of $[M-44]^+$ was also unique to these compounds. However, in the case of the terminal methyl ester (R_2 = CO–OCH_3) [Fig. 2(D)], the fragment ions generated by loss of terminal methyl ester (*m/z* 59) were not always detected, although the fragment ion of $[M-59]^+$ was clearly detected in compounds with the isopropyl group. In compounds with *tert*-butyl group, the abundance of the fragment ion formed by loss of *tert*-butyl group (*m/z* 56) was higher than that of $[M-59]^+$. The fragment ion of $[M-88]^+$, serially generated by loss of *m/z* 32 after desorption of tert-butyl group, was detected.

ESI-MS spectra

ESI-MS spectra were obtained at the exact mass using time of flight mass spectrometer. The spectra suggested the molecular information owing to soft ionization and varied among four fragmentor voltages. The abundance of $[M+H]^+$ was high in fragmentor voltage of 100 V, while the abundance of $[M+Na]^+$ was high in that of 350 V. In addition, the fragment information was also obtained in voltage from 100 to 350 V, although the number of ions was less than the EI-MS spectra. Four spectra of ADB-PINACA are shown in Fig. 4. The ion at *m/z* 345.2280 in Fig. 4(A) corresponds to $[M+H]^+$ ($C_{19}H_{29}N_4O_2^+$, *m/z* 345.2285), and the ion at *m/z* 367.2098 in Fig. 4(D) corresponds to $[M+Na]^+$ ($C_{19}H_{28}N_4O_2Na^+$, *m/z* 367.2104). All remaining ESI-MS spectra in voltage of 200 V are shown in Fig. S2.

The main fragment ions in ESI-MS were formed similarly to EI-MS by the cleavage of the amide group adjacent to the *N*-hetero rings. The peak of amide cleavage was prominent especially in 200 and 300 V. The ions at *m/z* 215.1184 and 215.1179, equivalent to $[M-129.1033]^+$, indicate the amide cleavage in Fig. 4(B) and (C), respectively. The fragment ion of $[M-16.0193]^+$ was formed by loss of NH_2 (*m/z* 16.0187) for compounds with the terminal amide (R_2 = CO–NH_2 in Table 1). For example, the ion at *m/z* 328.2022, equivalent to 328.2020 ($C_{19}H_{26}N_3O_2^+$), represents the fragment ion formed by loss of NH_2 in ADB-PINACA (Fig. 4(B)). However, the fragment ion formed by loss of terminal amide (*m/z* 44.0136) was not always detected unlike in the case of EI-MS. The fragment ion of $[M-44.0142]^+$ was generated for the compounds with the

Journal of MASS SPECTROMETRY

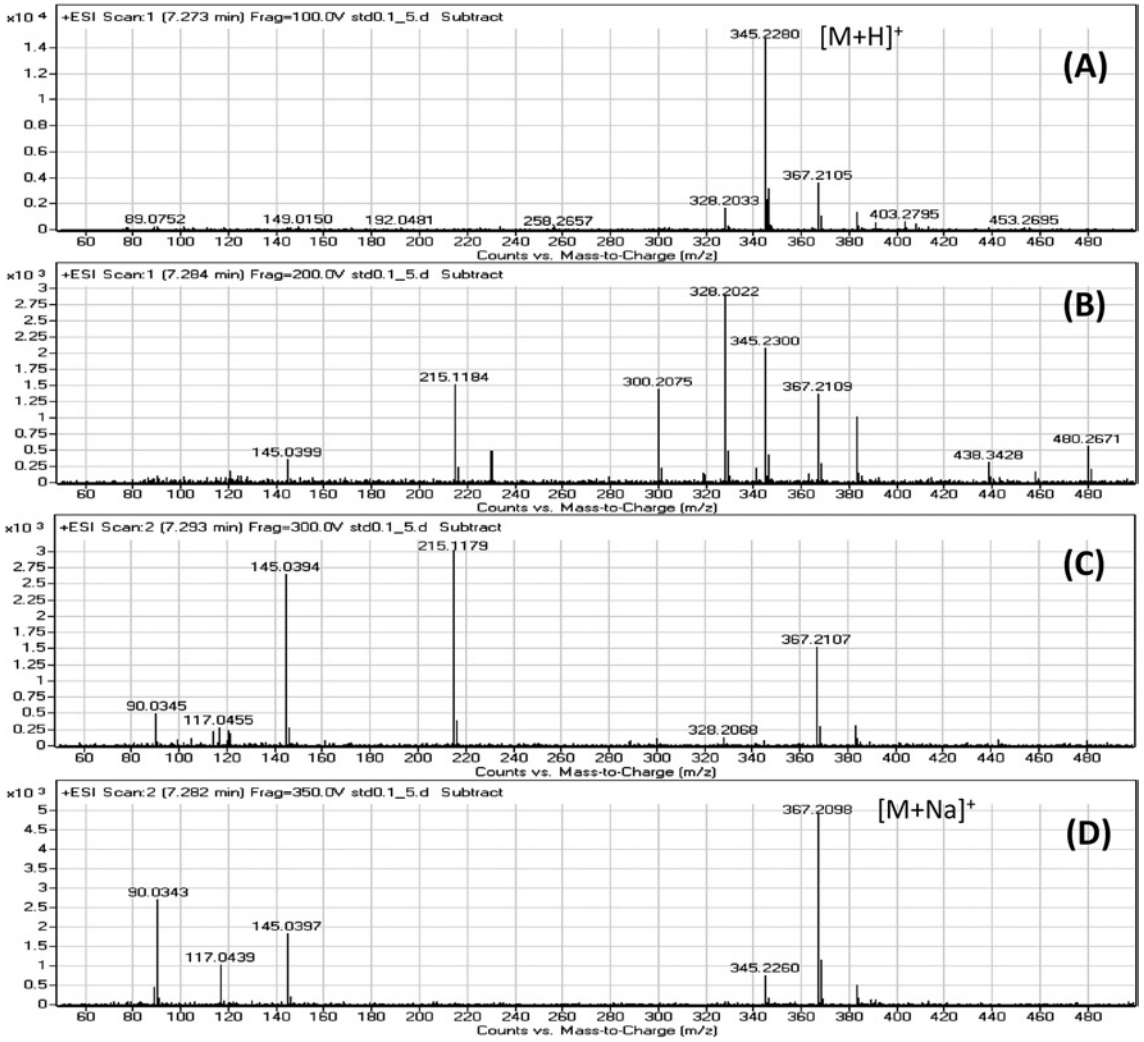


Figure 4. ESI-MS spectra of ADB-PINACA. Fragmentor voltage: (A) 100, (B) 200, (C) 300, (D) 350 V.

indazole ring as shown in Fig. 4(B). Meanwhile, the compounds with the indole ring, such as ADBICA and 5F-ABICA, are more likely not to detect the fragment ion of $[M\text{-}44.0142]^+$ as far as we investigate in this study. This means that the charge distribution of whole chemical structure also has an important part in the fragment pattern for ESI-MS, because there is a sufficient distance between the terminal amide group and the *N*-hetero ring. The fragment ion of $[M\text{-}59.0139]^+$ was formed by loss of the terminal ester for most compounds with the terminal methyl ester ($R_2 = CO\text{–}OCH_3$, *m/z* 59.0133).

Table 2. Characteristic fragment ions of the analytes

X [a]	R_1 [a]	R_2 [a]	*m/z*			
			EI	ESI (100 V)	ESI (200 V, 300 V)	ESI [b] (350 V)
CH	$C(CH_3)_3$	CO–NH_2	M-44, M-73, M-129, M-129-(R_3) [a, c]	M+1.0073	M-16.0193, M-129.1033	M+22.9892, 144.0444, 116.0495, 89.0386
		CO–OCH_3	M-56, M-88, M-144, M-144-(R_3)	M+1.0073	M-59.0139, M-144.1030	M+22.9892, 144.0444, 116.0495, 89.0386
	$CH(CH_3)_2$	CO–NH_2	M-44, M-99, M-115, M-115-(R_3)	M+1.0073	M-16.0193, M-115.0877	M+22.9892, 144.0444, 116.0495, 89.0386
		CO–OCH_3	M-59, M-114, M-130, M-130-(R_3)	M+1.0073	M-130.0874	M+22.9892, 144.0444, 116.0495, 89.0386
N	$C(CH_3)_3$	CO–NH_2	M-44, M-73, M-129, M-129-(R_3)	M+1.0073	M-16.0193, M-44.0142, M-129.1033	M+22.9892, 145.0396, 117.0447, 90.0338
		CO–OCH_3	M-56, M-88, M-144, M-144-(R_3)	M+1.0073	M-59.0139, M-144.1030	M+22.9892, 145.0396, 117.0447, 90.0338
	$CH(CH_3)_2$	CO–NH_2	M-44, M-99, M-115, M-115-(R_3)	M+1.0073	M-16.0193, M-44.0142, M-115.0877	M+22.9892, 145.0396, 117.0447, 90.0338
		CO–OCH_3	M-59, M-114, M-130, M-130-(R_3)	M+1.0073	M-59.0139, M-130.0874	M+22.9892, 145.0396, 117.0447, 90.0338

The *m/z* value in ESI is calculated to four places of decimals.
[a]Refer to in Table 1.
[b]Except compounds with 4-fluorobenzyl group.
[c]Parentheses indicates the molecular weight of the substituted group.

In the fragmentor voltage of 350 V, the abundance of amide cleavage fragment ion bound to the R_3 group was low, and the fragment ion dissociated from the R_3 group was easily detected. When the compound had the indole ring, the fragment ions were located at *m/z* 144.0444 ($C_9H_6NO^+$), 116.0495 ($C_8H_6N^+$), and 89.0386 ($C_7H_5^+$). The compound with the indazole ring also had three fragment ions at *m/z* 145.0396 ($C_8H_5N_2O^+$), 117.0447 ($C_7H_5N_2^+$), and 90.0338 ($C_6H_4N^+$). Actually ADB-PINACA had fragment ions at *m/z* 145.0397, 117.0439, and 90.0343 as shown in Fig. 4(D). Interestingly, for compounds with 4-fluorobenzyl group in the R_3 group, the previously mentioned three ions were not detected differently from the other compounds with fluorine moiety. Instead, two fragment ions were generated at *m/z* 109.0448 ($C_7H_6F^+$) and 83.0292 ($C_5H_4F^+$). This phenomenon is probably because of the stability of ionized 4-fluorobenzyl group. The fragmentation for ESI-MS is profoundly affected by both the ease of ionization and the site of ionization.

Estimation of chemical structures by both EI-MS spectra and ESI-MS spectra

Table 2 shows a summary for characteristic fragmentation of synthetic cannabinoids with the isopropyl group and the *tert*-butyl group using GC/MS and LC/MS. These fragment ions indicates the difference not only in the substituted group but also in the basic structure such as the *N*-hetero ring, which are useful for estimating the chemical structures. However, the fragmentation did not occur with absolute regularity, especially in ESI-MS spectra, and a substance-specific ion was observed. The substance-specific ion can be also useful for identifying the compound.

Concluding remarks

In this paper, a fragmentation of synthetic cannabinoids with an isopropyl group and a *tert*-butyl group was investigated using EI-MS and ESI-MS. The main fragment ions for both EI-MS and ESI-MS were formed by the cleavage of the amide group adjacent to the *N*-hetero rings. The detailed fragmentation analysis by multiple ionization techniques was efficient to distinguish the basic structure and the substituted group of compounds. The proposed fragment pattern is applicable to estimate the chemical structure of unknown compounds.

Finally, new designer drugs including synthetic cannabinoids have been created worldwide. Most compounds have a basic skeleton similar to controlled substances, which suggests that further studies of fragmentation analysis for each skeleton are required.

References

[1] R. Kikura-Hanajiri, N. Uchiyama, Y. Goda. Survey of current trends in the abuse of psychotropic substances and plants in Japan. *Leg Med* **2011**, *13*, 109.
[2] M. Mazzarino, X. Torre, F. Botre. A liquid chromatography–mass spectrometry method based on class characteristic fragmentation pathways to detect the class of indole-derivative synthetic cannabinoids in biological samples. *Anal Chim Acta* **2014**, *837*, 70.
[3] A. Namera, A. Nakamoto, T. Saito, M. Nagao. Colorimetric detection and chromatographic analyses of designer drugs in biological materials: a comprehensive review. *Forensic Toxicol* **2011**, *29*, 1.
[4] M. Kusano, K. Zaitsu, H. Nakayama, J. Nakajima, K. Hisatsune, T. Moriyasu, S. Matsuta, M. Katagi, H. Tsuchihashi, A. Ishii. Positional isomer differentiation of synthetic cannabinoid JWH-081 by GC-MS/MS. *J Mass Spectrom* **2015**, *50*, 586.
[5] L. M. Huppertz, S. Kneisel, V. Auwaerter, J. Kempf. A comprehensive library-based, automated screening procedure for 46 synthetic cannabinoids in serum employing liquid chromatography-quadrupole ion trap mass spectrometry with high-temperature electrospray ionization. *J Mass Spectrom* **2014**, *49*, 117.
[6] S. Matsuta, M. Katagi, H. Nishioka, H. Kamata, K. Sasaki, N. Shima, T. Kamata, A. Miki, M. Tatsuno, K. Zaitsu, K. Tsuboi, H. Tsuchihashi, K. Suzuki. Structural characterization of cathinone-type designer drugs by EI mass spectrometry. *Jpn J Forensic Sci Technol* **2014**, *19*, 77.
[7] D. Zuba. Identification of cathinones and other active components of 'legal highs' by mass spectrometric methods. *Trends Anal Chem* **2012**, *32*, 15.
[8] Y. Xing, X. Xu, B. Xu, H. Lei, Y. Xing, X. Liu, Q. Ma. Study on the mass fragmentation pathway of the synthetic cannabinoids JWH-018 and JWH-073. *Int J Mass Spectrom* **2015**, *379*, 139.
[9] F. W. McLafferty. Mass spectrometric analysis: Molecular rearrangements. *Anal Chem* **1959**, *31*, 82.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.