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ORIGINAL ARTICLE

Identification and quantitation of 5-fluoro-ADB-PINACA and MAB-CHMINACA in dubious herbal products

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Abstract Police officials brought three different packages of herbal blends, with brand names "AL 37", "AP 31", and "GM sapphire", to our laboratory for drug testing. Using our in-house high-resolution mass spectrometric analysis, we were able to estimate the presence of 5-fluoro-ADB-PINACA and MAB-CHMINACA in them without their reference standards. After obtaining the reference standards, we compared the mass spectra of the extracts of the herbal blends with those of the reference standards using both gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry. The mass spectra of the herbal blend extracts coincided with those of the reference standards, disclosing the presence of 5-fluoro-ADB-PINACA in "AL 37" and "AP 31", and MAB-CHMINACA in "GM sapphire". We then quantitated the concentrations of both compounds in the herbal blends using the standard addition method. The concentrations of 5-fluoro-ADB-PINACA were 19.4 ± 0.55 and 19.0 ± 0.47 mg/g (mean ± standard deviation of triplicate determinations) for herbal product brands "AL 37" and "AP 31", respectively, and that of MAB-CHMINACA was 133 ± 4.5 mg/g for the "GM sapphire" herbal product. To our knowledge, this is the first study to demonstrate the identification and quantitation of the newest synthetic cannabinoids 5-fluoro-ADB-PINACA and MAB-CHMINACA in herbal blend products.

Keywords 5-Fluoro-ADB-PINACA · 5-Fluoro-AB-PINACA · MAB-CHMINACA · AB-CHMINACA · Herbal products · Synthetic cannabinoid · LC–MS–MS

Introduction

In 2013, Uchiyama et al. [1] disclosed the presence of a new and unique cannabimimetic designer drug in herbal products, with a structure in which an indazole ring was connected to a carbamyl moiety; the amino group was bound to a "horned" isopropyl methyl moiety that was further bound to another carbamyl moiety. They named it AB-PINACA. In the same year, Uchiyama et al. [2] also identified a new drug very similar to AB-PINACA in structure, in which the isopropyl methyl moiety found in AB-PINACA was replaced by a *t*-butyl methyl moiety; they named this drug ADBICA. In 2014, the same group [3] reported the identification of 5-fluoro-AB-PINACA and AB-CHMINACA present in illegal products, which can be produced by fluorination of the 5"-terminal of the 1-pentyl chain and by the replacement of the 1-pentyl chain with 1-cyclohexylmethyl moiety, respectively. In the present article, we report identification and quantitation of 5-fluoro-ADB-PINACA and MAB-CHMINACA (synonym: ADB-CHMINACA) as new synthetic cannabinoids in herbal blend products. Their structures are very similar to those of 5-fluoro-AB-PINACA and AB-CHMINACA, respectively, the only difference of which is that the isopropyl methyl moiety of 5-fluoro-AB-PINACA and AB-CHMINACA are replaced by the *t*-butyl methyl moiety in the new compounds (Fig. 1).

A. Wurita and K. Hasegawa contributed equally to this work.

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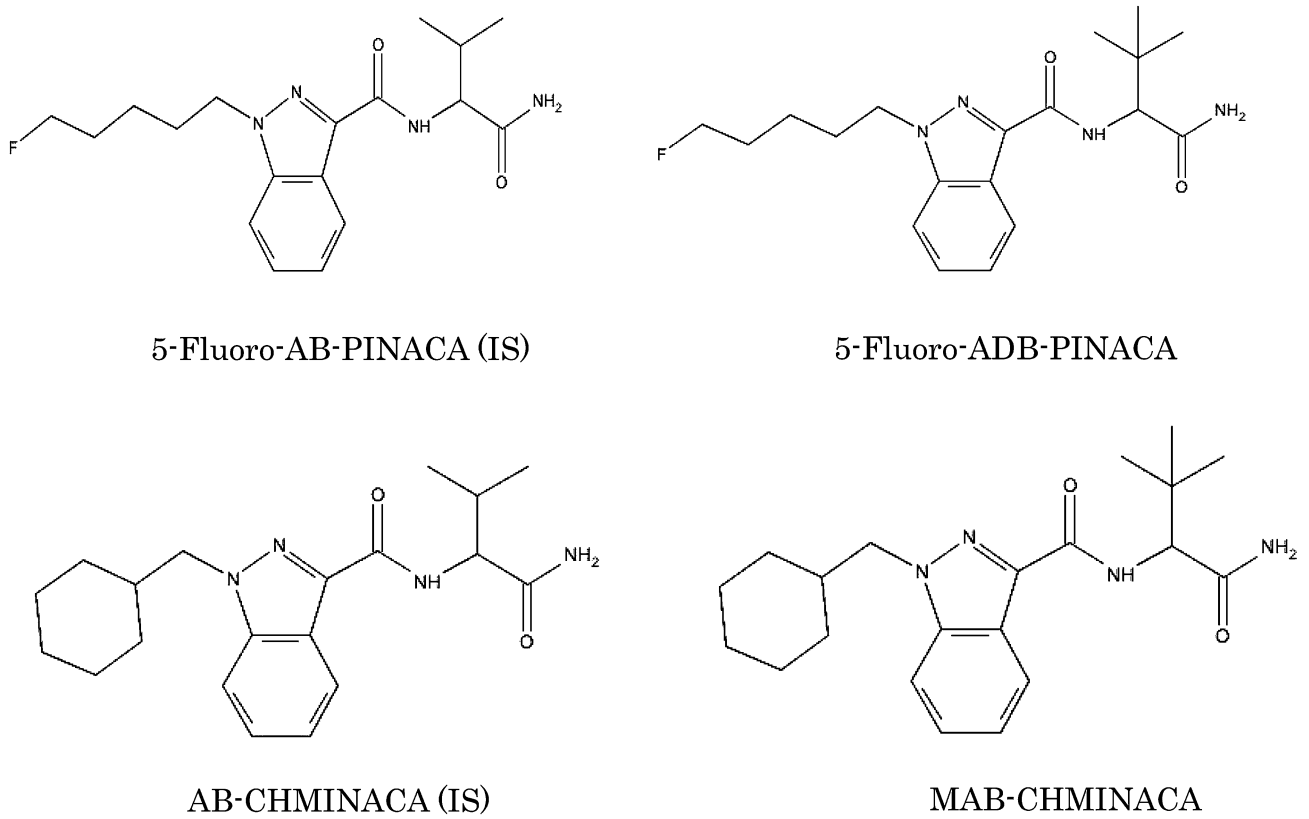

Fig. 1 Structures of 5-fluoro-ADB-PINACA, MAB-CHMINACA, and internal standards (ISs) used in this study

Case history

Three herbal blends were brought to our laboratory for analysis, together with a cadaver. At the time of autopsy of the cadaver, the primary cause of death was considered to be 5-fluoro-ADB poisoning, as this compound was detected in the stomach contents and five solid tissues, and 49.2 ± 2.46 mg/g of the compound was detected in "GM sapphire", one of the three herbal products [4]. At that time, however, we noticed that some unknown peaks other than 5-fluoro-ADB were present in total ion chromatograms of gas chromatography–mass spectrometry (GC–MS) for the extracts of the three herbal blends. We consulted the Cayman Spectral Library [5], but it did not suggest a compound. Shortly after the autopsy, we reexamined the extracts for the presence of other drug(s) in the herbal products using accurate mass spectrometry (MS) on a matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometer. The instrument used was the QSTAR Elite Hybrid quadrupole TOF mass spectrometer (AB SCIEX, Framingham, MA, USA). Details of the MALDI-TOF–MS procedure are described in our previous report [6]. The MALDI tandem quadrupole-TOF mass spectra strongly suggested the presence of 5-fluoro-ADB-PINACA and MAB-CHMINACA in the extracts. This was the starting point of the present study.

Materials and methods

Materials

Three silver-colored packages of herbal blends were found near the deceased in his room in a house owned by his mother, and were brought to our laboratory for analysis. The brand names, handwritten with a maker pen, were "AL 37", "AP 31", and "GM sapphire". The reference standards 5-fluoro-ADB-PINACA, MAB-CHMINACA, 5-fluoro-AB-PINACA (internal standard, IS), and AB-CHMINACA (IS) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). The structures of the target compounds and respective ISs are shown in Fig. 1.

Other common chemicals used were of the highest purity available commercially.

Pretreatment procedure

Extraction of synthetic cannabinoids from herbal debris was performed according to the method previously

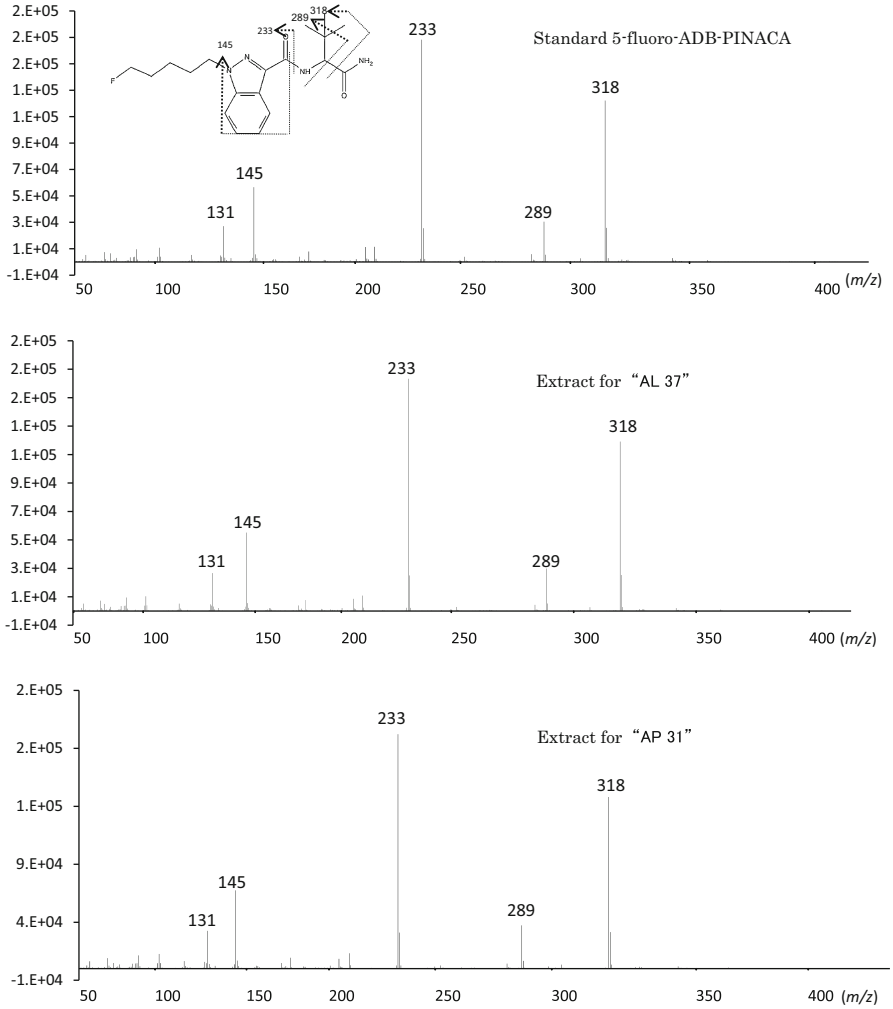

Fig. 2 Mass spectra of the reference standard of 5-fluoro-ADB-PINACA and the acetonitrile extracts of herbal blend products "AL 37" and "AP 31" obtained by gas chromatography–mass spectrometry (GC–MS) with electron ionization (EI) together with the probable fragmentation mode

described [7], with modification. We changed the methanol extraction medium to acetonitrile, as methanol has been reported to cause thermal degradation of synthetic cannabinoids when GC–MS is employed for analysis [8]. Ten milligrams each of the herbal debris was placed in a centrifuge tube containing 1.0 ml of acetonitrile, sonicated for 10 min, and centrifuged at 10,000 rpm for 2 min. The supernatant layer was decanted into a test tube. The acetonitrile extract solution was diluted 100-fold with acetonitrile for the "AL 37" and "AP 31" products and 1,000-fold for the "GM sapphire" product. To 1.0 ml each of the diluted acetonitrile extract solution, 1.0 μg each of IS (for analysis of 5-fluoro-ADB-PINACA, 5-fluoro-AB-PINACA; for analysis of MAB-CHMINACA, AB-CHMINACA) dissolved in 10 μl of acetonitrile, with or without an appropriate amount of the reference standard of 5-fluoro-ADB-PINACA or MAB-CHMINACA, was added and the mixture gently shaken. A 1.0-μl aliquot of the solution was injected into a GC–MS instrument; a 3.5 μl aliquot of the same solution was injected into an LC–MS–MS instrument.

GC–MS conditions

The GC–MS instrument used was an Agilent 6850 Series gas chromatograph connected to 5975 Series mass spectrometer (Agilent, Santa Clara, CA, USA). GC conditions were as follows: separation column, Agilent HP-5ms fused silica capillary (30 m × 0.25 mm i.d., 0.25 μm film thickness); injector temperature, 250 °C; interface temperature, 280 °C; injection mode, splitless; injection volume, 1 μl; carrier gas (He) pressure, 151 kPa; oven temperature program, initial temperature at 60 °C (2-min hold) followed by ramp at 20 °C/min up to 300 °C (10-min hold). MS conditions were the following: ion source temperature, 230 °C; ionization mode, electron ionization (EI) at 70 eV; emission current, 35 μA; detection gain, 1,118 V; identification, scan mode; scan range, *m/z* 50–400; and scan speed, 2.86 scans/s.

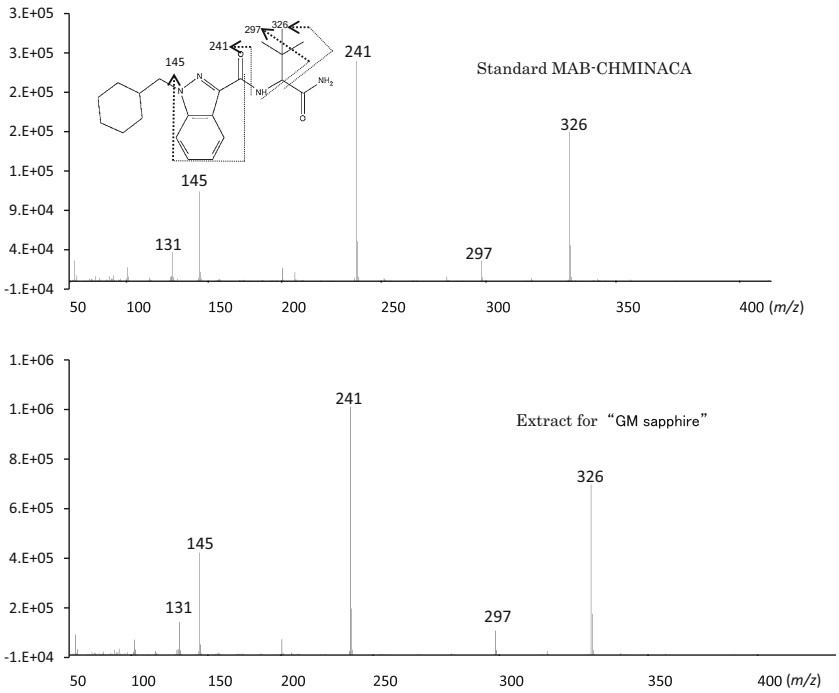

Fig. 3 Mass spectra of the reference standard of MAB-CHMINACA and an acetonitrile extract of the "GM sapphire" herbal blend product obtained by GC–MS with EI together with the probable fragmentation mode

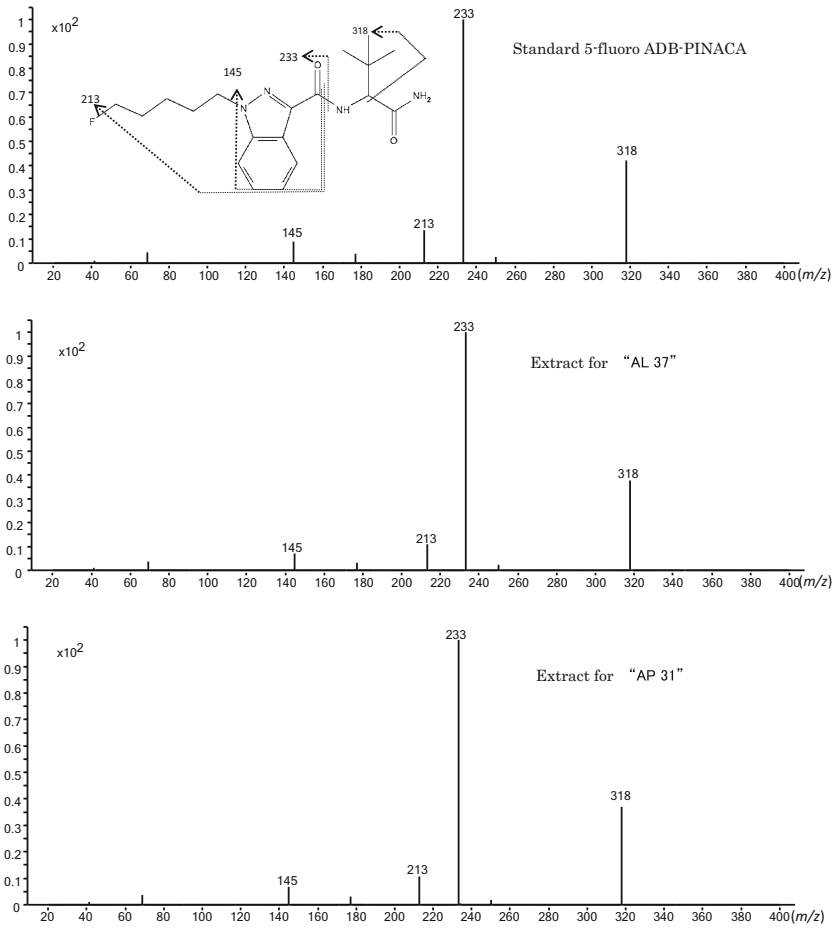

Fig. 4 Product ion mass spectra of the reference standard 5-fluoro-ADB-PINACA and acetonitrile extracts of herbal blend products "AL 37" and "AP 31" obtained by liquid chromatography–tandem mass spectrometry (LC–MS–MS) together with the probable fragmentation mode

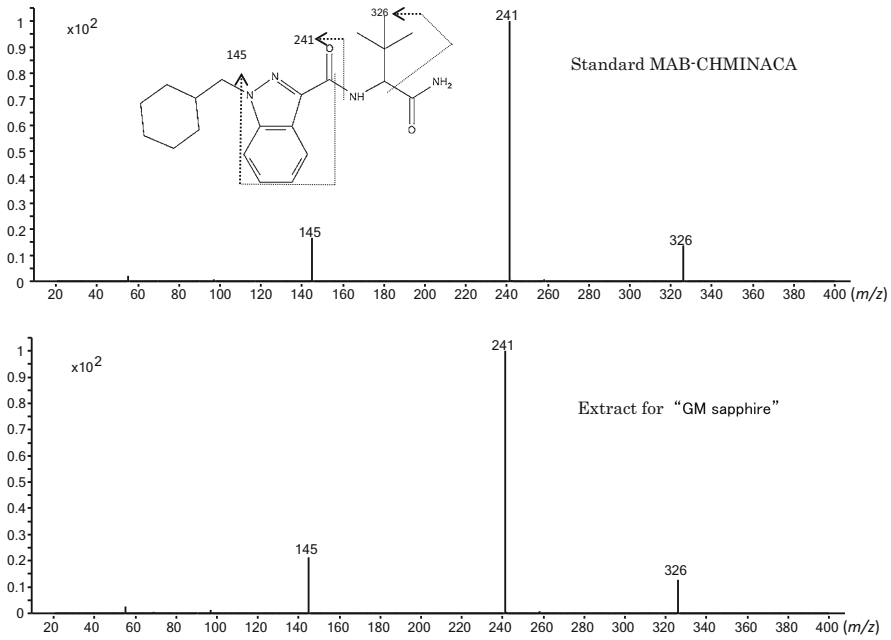

Fig. 5 Product ion mass spectra of the reference standard MAB-CHMINACA and an acetonitrile extract of the "GM sapphire" herbal mixture obtained by LC–MS–MS together with the probable fragmentation mode

LC–MS–MS conditions

LC–MS–MS with electrospray ionization (ESI) was conducted on an Agilent 1200 Series LC-SL system containing a micro degasser and a high-performance autosampler, which was connected to a 6460 Triple Quad LC/MS tandem MS instrument (Agilent). For LC separation, a ZORBAX Eclipse Plus C18 column (100 × 2.1 mm internal diameter, particle size 1.8 µm; Agilent) was used. The LC conditions were as follows: injection volume, 3.5 µl; flow rate, 0.25 ml/min; elution mode, gradient with 10 mM ammonium formate/0.1 % formic acid in distilled water (A) and acetonitrile (B) from 60 % A/40 % B to 100 % B over 15 min, followed by isocratic elution with the final solvent composition for 10 min. The column and autosampler were operated at room temperature.

The tandem MS conditions were as follows: interface, ESI mode; polarity, positive ion mode; ion source temperature, 320 °C; ion source voltage, 500 V; quantitation, selected reaction monitoring (SRM) mode using peak area; ion transitions, *m/z* 363 → 318 for 5-fluoro-ADB-PINACA, *m/z* 349 → 304 for 5-fluoro-AB-PINACA (IS), *m/z* 371 → 241 for MAB-CHMINACA, and *m/z* 357 → 241 for AB-CHMINACA (IS); fragmentor voltage and collision energy, 120 and 9 V for 5-fluoro-ADB-PINACA, 120 and 9 V for 5-fluoro-AB-PINACA, 120 and 25 V for MAB-CHMINACA, and 120 and 21 V for AB-CHMINACA, respectively.

Data acquisition, peak integration, and calculation were performed with a computer workstation (Agilent MassHunter, Revision Acquisition B. 02. 01, Qualification B. 03. 01SP2, and Quantification B. 04. 00).

Standard addition method

The standard addition method is frequently used for analysis by atomic absorption spectroscopy [9]. Because this method does not require a blank matrix, and completely overcomes the matrix effects, we use this method for analysis of compound(s) existing in matrices with different properties [4, 7, 10–14]. In this study, because we were dealing with three herbal blends, all with properties that were likely quite different, we also used the standard addition method, although each acetonitrile extract solution was highly diluted. The principle of the method and examples of the standard addition calibration curve were discussed in detail in our previous report [15].

Results and discussion

As shown in Fig. 2, the mass spectra obtained from the major total ion chromatogram peaks for the extracts of herbal brands "AL 37" and "AP 31" coincided with that of the reference standard 5-fluoro-ADB-PINACA by GC–MS. For the herbal brand "GM sapphire", the mass spectrum of its extract completely coincided with that of the reference standard MAB-CHMINACA by GC–MS, as shown in Fig. 3.

We also identified the above-referenced compounds in the herbal extracts by comparing product ion mass spectra of the extracts with those of the reference standards using LC–MS–MS. The spectra obtained from the herbal blends "AL 37" and "AP 31" coincided with that of the reference standard 5-fluoro-ADB-PINACA, as shown in Fig. 4. The

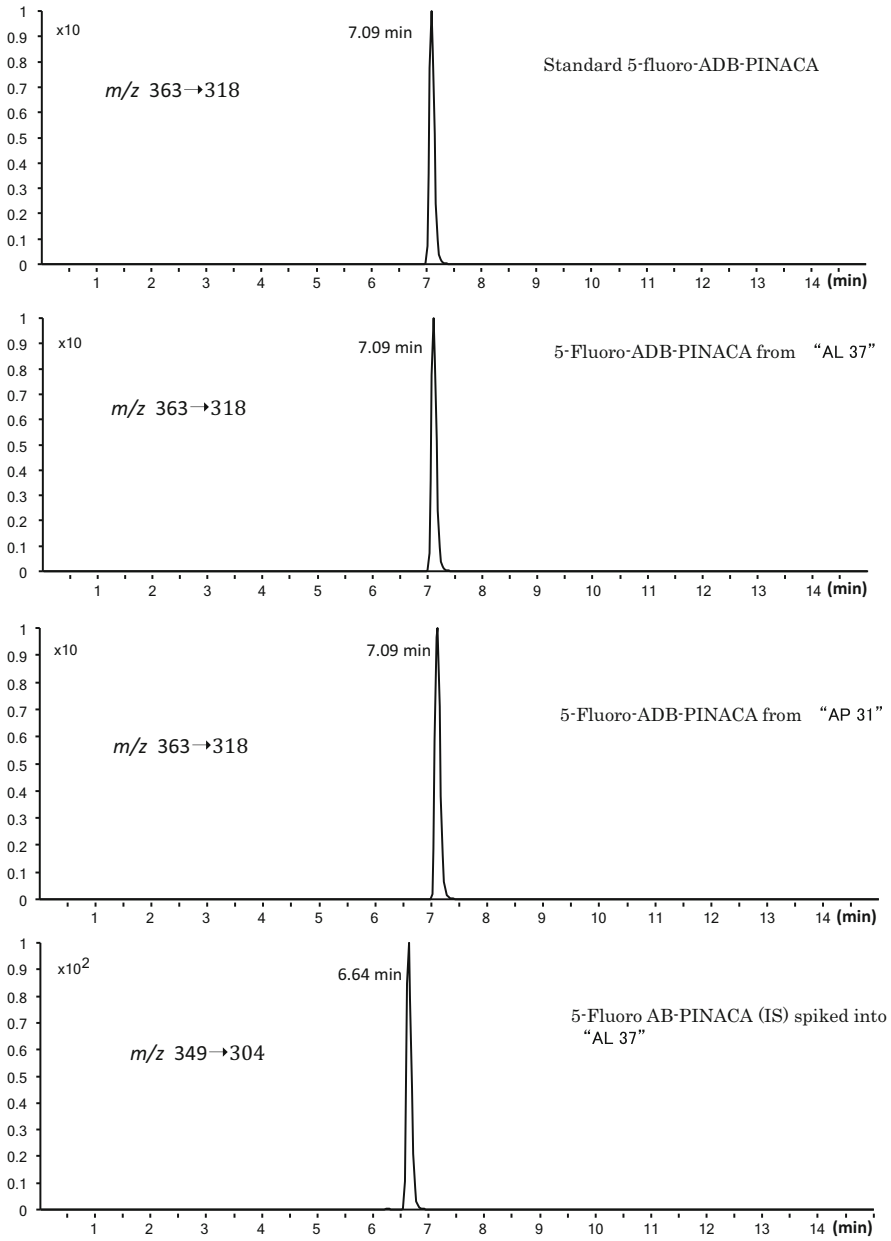

Fig. 6 Selected reaction monitoring (SRM) chromatograms using LC–MS–MS for the reference standard 5-fluoro-ADB-PINACA, acetonitrile extracts of herbal blend products "AL 37", "AP 31", and 5-fluoro-AB-PINACA (IS) spiked into the extract of "AL 37"

spectrum obtained from the herbal blend "GM sapphire" completely coincided with that of reference standard MAB-CHMINACA, as shown in Fig. 5.

We confirmed that 5-fluoro-ADB-PINACA was identifiable in "AL 37" and "AP 31", but not in "GM sapphire"; MAB-CHMINACA was identifiable only in "GM sapphire", and not in "AL 37" or "AP 31".

In comparing the mass spectra by GC–MS with those by LC–MS–MS, we noticed the appearance of some fragment ions in common between GC–MS and LC–MS–MS. The ions at *m/z* 318, 233, and 145 of 5-fluoro-ADB-PINACA appeared in both GC–MS and LC–MS–MS, and those at *m/z* 326, 241, and 145 of MAB-CHMINACA also appeared in both.

In the next step, we quantitated the concentrations of 5-fluoro-ADB-PINACA and MAB-CHMINACA in the herbal blends by LC–MS–MS with the standard addition method. Figure 6 shows SRM chromatograms for 5-fluoro-ADB-PINACA in the "AL 37" and "AP 31" products and 5-fluoro-AB-PINACA spiked into "AL 37". For each chromatogram, an intense sharp peak appeared, with no impurity peak. The peaks of 5-fluoro-ADB-PINACA and 5-fluoro-AB-PINACA (IS) appeared at 7.09 and 6.64 min, respectively.

For the SRM chromatograms for MAB-CHMINACA, a good-shaped peak also appeared without any impurity peak. The retention times of MAB-CHMINACA and AB-CHMINACA (IS) were 8.90 and 8.10 min, respectively (Fig. 7).

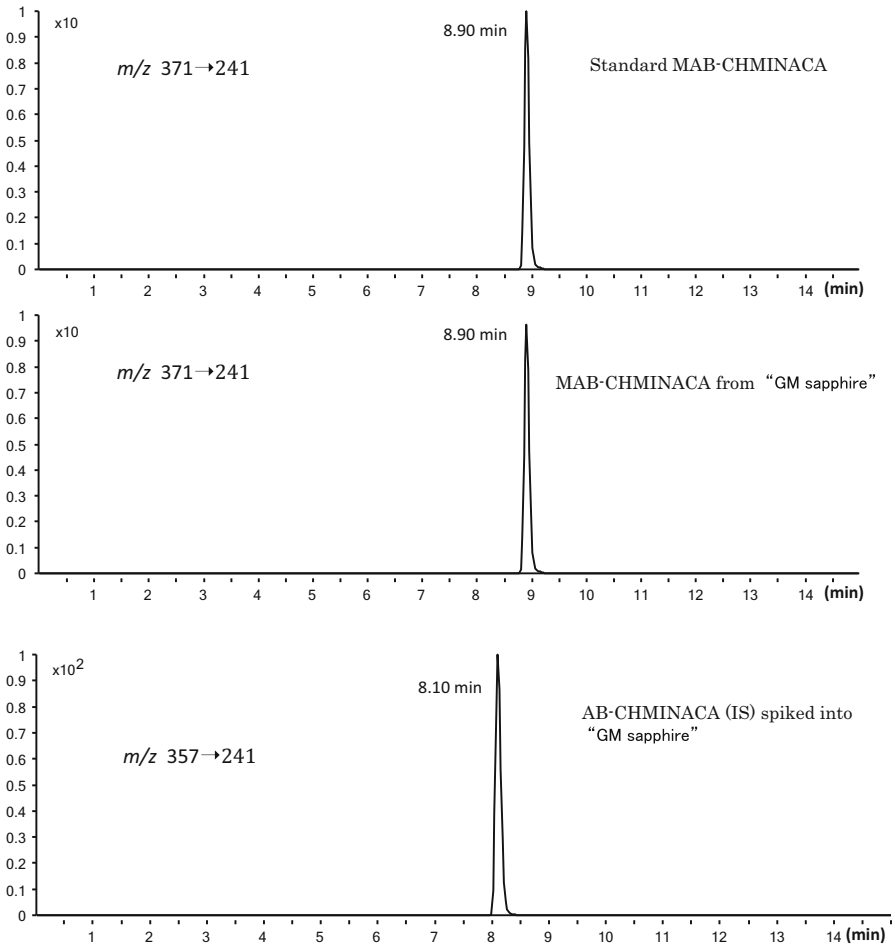

Fig. 7 SRM chromatograms using LC–MS–MS for the reference standard MAB-CHMINACA, an acetonitrile extract of herbal mixture product "GM sapphire", and AB-CHMINACA (IS) spiked into the extract of "GM sapphire"

Table 1 Standard addition calibration equations for the two drugs in three herbal mixture products

Analyte	Specimen	Equation[a]	Correlation coefficient(r)
5-Fluoro-ADB-PINACA	"AL 37"	$y = 0.0437x + 0.845$	0.997
	"AP 31"	$y = 0.0437x + 0.831$	0.996
MAB-CHMINACA	"GM sapphire"	$y = 0.00689x + 0.915$	0.998

[a] If y equals 0, the preexisting concentration (x) in mg/g can be calculated as a negative value.

The standard addition calibration equations together with correlation coefficient values according to the drugs and herbal mixture products are shown in Table 1. The correlation coefficient values obtained for all equations were greater than 0.995. Using these equations, we found that the levels of 5-fluoro-ADB-PINACA were 19.4 ± 0.55 mg/g ($n = 3$) for "AL 37" and 19.0 ± 0.47 mg/g ($n = 3$) for "AP 31"; the level of MAB-CHMINACA in "GM sapphire" was 133 ± 4.5 mg/g ($n = 3$).

In our previous article [4], we reported the identification and quantitation of 5-fluoro-ADB in autopsy specimens and herbal products. We focused much attention in the study on 5-fluoro-ADB, as some specialists had noted that 5-fluoro-ADB caused unconsciousness followed by cardiopulmonary arrest shortly after smoking an herbal product containing this compound. In fact, since late September 2014, there have been about ten deaths in Japan caused by 5-fluoro-ADB (Kikura-Hanajiri, personal communication). It is regarded as the most potent, and thus dangerous, synthetic cannabinoid ever known. Although at the time of the autopsy we noted the appearance of peaks other than 5-fluoro-ADB in the herbal mixtures, we failed to identify the peaks. Subsequent efforts to identify them have led us to the present fruitful results in disclosing 5-fluoro-ADB-PINACA and MAB-CHMINACA in the herbal blends. Given that the "GM sapphire" contained 49.2 ± 2.56 mg/g of 5-fluoro-ADB [4], it is true that the deceased subject reported in the previous article [4] smoked at least the "GM sapphire" herbal blend, as 5-fluoro-ADB was detected in his stomach contents and five solid tissue specimens. This implies that the deceased inhaled not only 5-fluoro-ADB, but also MAB-CHMINACA, given the coexistence of 5-fluoro-ADB and MAB-

CHMINACA in the "GM sapphire" herbal blend as evidenced in the present study. It is likely that MAB-CHMINACA in this case acted to enhance the toxicity of 5-fluoro-ADB [4].

Conclusions

In the present study, we identified and quantitated 5-fluoro-ADB-PINACA and MAB-CHMINACA in three herbal blend products, which had also been involved in the previous study for detection of 5-fluoro-ADB in a poisoning case [4] and stored in our laboratory. 5-Fluoro-ADB, 5-fluoro-ADB-PINACA, and MAB-CHMINACA are all analogs of indazole carboxamide synthetic cannabinoids. The most characteristic structure of this class is the presence of *t*-butyl methyl moiety next to the carbamyl group. The contribution of *t*-butyl methyl moiety to the toxicity of this class of compounds remains to be explored. To our knowledge, this is the first scientific description of the presence of 5-fluoro-ADB-PINACA and MAB-CHMINACA in dubious herbal products.

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

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