—Technical Note—

Elucidation of the fluorine substitution position on the phenyl ring of synthetic cannabinoids by electron ionization-triple quadrupole mass spectrometry

Takaya Murakami¹, Yoshiaki Iwamuro¹, Reiko Ishimaru¹, Satoshi Chinaka¹,
Ippei Noda², Shuhei Higashibayashi², and Nariaki Takayama¹

¹*Forensic Science Laboratory, Ishikawa Prefectural Police H.Q.,
1-1 Kuratsuki, Kanazawa, Ishikawa 920-8553, Japan*

²*Institute for Molecular Science,
38 Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585, Japan*

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We present herein a practical methodology for elucidating the *o*-, *m*-, or *p*-fluorine substitution pattern of indazole-type synthetic cannabinoids containing a fluorobenzyl group at the N-1 position and a carbonyl group at the C-3 position via electron ionization-triple quadrupole mass spectrometry. We synthesized, as model compounds of the synthetic cannabinoids, the *o*-, *m*-, and *p*-fluorine positional isomers: 1-[1-(2-, 3-, and 4-fluorobenzyl)-1*H*-indazol-3-yl]ethanone (*o*-, *m*-, and *p*-FUBINAE). Mass spectral analyses showed that the three isomers differed significantly in the logarithmic values of the abundance ratios of the product ion at m/z 109 to the precursor ion at m/z 253 ($\ln(A_{109}/A_{253})$), following the order of *meta* < *ortho* < *para*. In addition, the relationships between $\ln(A_{109}/A_{253})$ and collision energy were linear with high correlation coefficients. Comparing the $\ln(A_{109}/A_{253})$ plots of the FUBINAE isomers versus collision energy with similar plots of AB-FUBINACA and its *o*- and *m*-fluorobenzyl isomers showed that the three AB-FUBINACA isomers behaved as the FUBINAE isomers did with the same fluorine substitution pattern on the phenyl ring. Moreover, other synthetic cannabinoids with a *p*-fluorobenzyl group (ADB-FUBINACA, FUB-AMB, FUB-APINACA, FUB-NPB-22, and FU-PX-2) also exhibited behavior similar to *p*-FUBINAE. These results indicated that the fluorine substitution position on the phenyl ring can be differentiated by collating the model compounds according to the logarithmic plots of their mass spectral abundance ratios as a function of the collision energy.

Key words: Synthetic cannabinoid, Model compound, Positional differentiation, Product ion spectrometry, Collision energy

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Introduction

Since the emergence of new classes of designer drugs, such as synthetic cannabinoids (SCs) and synthetic cathinones, many health and social concerns have arisen as a result of their abuse¹⁻³. In an effort to deter the use of such designer drugs, the Japanese Ministry of Health, Labor and Welfare has increased the number of controlled drugs ‘designated substances’ regulated under the Pharmaceutical and Medical Device Act (previously the Pharmaceutical Affairs Law) to 2,348 as of September 2016. Despite these increased restrictions, new designer drugs are continually being created with slight modifications to the molecular structures of regulated illicit drugs, thereby circumventing existing legal measures⁴. This requires forensic analysts to be even more meticulous when attempting to classify seized designer drugs as either legal or illegal.

Mass spectrometry is considered a powerful tool for identifying the molecular structures of organic compounds, including abused drugs⁵. Several research groups have reported differentiations of structural isomers based on differences in the fragment ions in full scan/product ion scan mass spectra⁶⁻¹². However, a group of SCs containing a fluorobenzyl group at the N-1 position, including *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (AB-FUBINACA)^{13,14}, *N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (ADB-FUBINACA)^{14,15}, methyl 2-[1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamido]-3-methylbutanoate (FUB-AMB; AMB-FUBINACA)¹⁶, and *N*-(1-adamantyl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (FUB-APINACA; FUB-AKB48)¹⁷, were difficult to distinguish from their fluorine positional isomers in both full scan and product ion scan mass spectra because few characteristic fragment ions were observed. Contributing to this challenge was the fact that the reference standards for the positional isomers were not

readily available on the market. These difficulties prompted us to develop a new method for the practical differentiation of these positional isomers. We previously reported a differentiation method for AB-FUBINACA and its fluorobenzyl isomers based on the differences of the abundance ratios of the product ions resulting from the dissociation reactivity between the indazole moiety and the fluorobenzyl group¹⁸. We expected this approach to also be applicable to the positional differentiation for indazole-type SCs containing a fluorobenzyl group at the N-1 position and a carbonyl group at the C-3 position collectively. In this study, we synthesized *o*-, *m*-, and *p*-fluorine positional isomeric model compounds of such SCs and investigated their product ion scan spectra using a similar technique to our previous study. We compared the logarithmic values of the mass spectral abundance ratios of the model compounds with those of several SCs.

Materials and Methods

Chemicals

1-(1*H*-indazole-3-yl) ethanone was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) 2-, 3-, and 4-Fluorobenzyl bromide were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (AB-FUBINACA), its *o*-fluorobenzyl and *m*-fluorobenzyl isomers, *N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (ADB-FUBINACA), methyl 2-[1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamido]-3-methylbutanoate (FUB-AMB), *N*-(1-adamantyl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (FUB-APINACA), quinolin-8-yl 1-(4-fluorobenzyl)-1*H*-indazole-3-carboxylate (FUB-NPB-22), *N*-(1-amino-1-oxo-3-phenylpropan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (FUPX-2) were purchased from Cayman Chemical (Ann Arbor, MI, USA). The standard solutions of the SCs (200 µg/mL) were prepared in methanol and were stored at -20°C. The working standard solutions (20 µg/mL) that

were injected into the mass spectrometer were prepared by diluting the stock solutions. An herbal mixture laced with AB-FUBINACA, packed in a plastic bag, was seized from a suspect. The herbal material (~ 10 mg) was extracted with 1 mL of methanol under ultrasonication for 10 min. After the extract was centrifuged at 3000 rpm for 1 min, the supernatant was filtered by an Ultrafree[®]-MC GV centrifugal filter unit with Durapore[®] PVDF 0.22 μ m membrane (Millipore, Bedford, MA, USA). The solution was diluted with methanol to a suitable concentration for instrumental analysis.

Synthesis of 1-[1-(2-, 3-, and 4-fluorobenzyl)-1*H*-indazol-3-yl]ethanone (*o*-, *m*-, and *p*-FUBINAE)

To a solution of 1-(1*H*-indazol-3-yl)ethanone (29.7 mg) in dry *N,N*-dimethylformamide (1.8 mL) under argon atmosphere was added 60% sodium hydride (11.4 mg) at room temperature. After stirring for 30 min, 4-fluorobenzyl bromide (34.5 μ L) was added. The mixture was stirred for 1 h and quenched by ammonium chloride aqueous solution. The mixture was extracted with ethyl acetate:hexane (1:1) 3 times. The combined organic extracts were dried over anhydrous sodium sulfate, filtered through Celite[®], and evaporated. The residue was purified by preparative thin-layer chromatography (Wakogel[®] B-5F (Wako Pure Chemical Industries, Ltd., Osaka, Japan), ethyl acetate:hexane=1:2) as the eluent to afford 1-[1-(4-fluorobenzyl)-1*H*-indazol-3-yl]ethanone (*p*-FUBINAE, 44.2 mg, 89%) as a colorless solid. 1-[1-(2-Fluorobenzyl)-1*H*-indazol-3-yl]ethanone (*o*-FUBINAE, 20.7 mg, 59%) and 1-[1-(3-fluorobenzyl)-1*H*-indazol-3-yl]ethanone (*m*-FUBINAE, 21.9 mg, 66%) were also prepared by the same method from 1-(1*H*-indazol-3-yl)ethanone (20 mg), respectively. The chemical structures were confirmed by proton nuclear magnetic resonance (¹H-NMR) spectroscopy. ¹H-NMR spectra (400 MHz, CDCl₃) were recorded on a JNM-ECS 400 (JEOL Ltd., Tokyo, Japan) at 23°C with

residual solvent peak at 7.24 ppm as an internal standard. The ¹H-NMR data for each synthesized compound are summarized below.

o-FUBINAE; ¹H NMR (CDCl₃) δ : 8.35 (1H, d, $J=7.9$ Hz), 7.47–7.35 (2H, m), 7.33–7.24 (2H, m), 7.11–7.00 (3H, m), 5.69 (2H, s), 2.72 (3H, s).

m-FUBINAE; ¹H NMR (CDCl₃) δ : 8.37 (1H, d, $J=7.9$ Hz), 7.41–7.25 (4H, m), 7.02–6.93 (2H, m), 6.88 (1H, d, $J=9.5$ Hz) 5.63 (2H, s), 2.77 (3H, s).

p-FUBINAE; ¹H NMR (CDCl₃) δ : 8.36 (1H, d, $J=8.0$ Hz), 7.40–7.27 (3H, m), 7.23–7.17 (2H, m), 7.02–6.95 (2H, m), 5.60 (2H, s), 2.72 (3H, s).

Mass spectrometry

Mass spectra were obtained by a SCION TQ triple quadrupole mass spectrometer (Bruker Daltonics, Billerica, MA, USA) with a BR-5ms fused-silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness; Bruker Daltonics, Billerica, MA, USA). The inlet temperature was set at 200°C. The injection mode was splitless and the sample injection volume was 1 μ L. The carrier gas was high-purity helium of six nine-grade at a constant column flow rate of 45.1 cm/sec. The temperature program for full scan mode consisted of an initial temperature at 80°C held for 1 min, ramped up to 300°C at 10°C/min followed by a hold at 300°C for 15 min. The temperature program for product ion scan mode consisted of an initial temperature at 200°C held for 1 min, ramped up to 300°C at 10°C/min followed by a hold at 300°C for 10 min. The transfer line was maintained at 280°C. The mass spectrometer was operated in EI mode at 70 eV using an ion-source temperature of 230°C. The acquisition of full scan mode was in the range of m/z 40–600. The acquisition of product ion scan mode was in the range of m/z 40–253 for the precursor ion at m/z 253. Argon gas pressure on collision-induced dissociation (CID) reaction was set at 1.5 mTorr. In order to obtain an acceptable quality spectrum, the mass spectrometer was tuned according to the instrument protocol as needed. All the analyses described below in our present method should be

completed in as short a period of time as possible using the same instrumental condition, since the given collision energy potentially changes slightly due to instrumental mechanical fluctuations.

Statistical calculations

Statistical analyses were performed using BellCurve for Excel ver. 2.02 (Social Survey Research Information Co., Ltd., Tokyo, Japan), which is add-in software to Microsoft Excel 2010. The homogeneity of variances was calculated by Bartlett's test to determine if obtained data were normally distributed (parametric, $p > 0.05$) or not (nonparametric, $p < 0.05$). If the data were parametric, one-way analysis of variance (ANOVA) followed by multiple pairwise comparisons as post hoc analysis using Tukey's test was carried out. For the nonparametric data, nonparametric ANOVA (Kruskal-Wallis test) followed by multiple pairwise comparisons using Steel-Dwass test was carried out.

Results and Discussion

Gas chromatography

Gas chromatographic separation of the *o*-, *m*-, and *p*-FUBINAE isomers was achieved using a capillary column of 30 m $\times$ 0.25 mm i.d. and 0.25 μ m film thickness of the less polar stationary phase, 5% diphenyl/95% dimethyl arylene siloxane (BR-5ms) under the temperature program for full scan mode (Fig. 1). The three isomers eluted in the order of *o*-, *m*-, *p*-FUBINAE. The calculated retention indices were 2158 for *o*-FUBINAE, 2170 for *m*-FUBINAE, and 2183 for *p*-FUBINAE.

Full scan mass spectra of *o*-, *m*-, and *p*-FUBINAE isomers

Figure 2 shows the full scan mass spectra of the *o*-, *m*-, and *p*-FUBINAE isomers. The three isomers gave identical fragment ions. Major fragment ions were observed at m/z 43, 83, 109, 225, 253, and 268. The proposed electron ionization fragmentation pathways are shown in Fig. 3. The m/z 268 ion is the molecular radical cation of the analyte resulting from initial ionization of the indazole nitrogen (Fig. 3a), the

Fig. 1 Total ion current chromatogram for a mixture of *o*-, *m*-, and *p*-FUBINAE.

Fig. 2 Full scan mass spectra of *o*-, *m*-, and *p*-FUBINAE.

Fig. 3 Proposed electron ionization fragmentation pathways of *o*-, *m*-, and *p*-FUBINAE.

carbonyl oxygen (Fig. 3b), or the fluorobenzene carbon (Fig. 3c) in accordance with classical mass spectral interpretation^{19,20}. The *m/z* 253 ion could originate from the nitrogen (Fig. 3d) or the carbonyl oxygen (Fig. 3e), resulting in the loss of a methyl radical by homolytic cleavage between the carbonyl and methyl carbons¹⁹. The mechanisms by which the fragment ions at *m/z* 268 (Fig. 3a and 3b) and *m/z* 253 (Fig. 3d and 3e) are formed are essentially the same because they are resonance structures. The *m/z* 225 ion is the carbenium cation generated by elimination of carbon monoxide from the *m/z* 253 ion. The *m/z* 109 ion, which is the base peak, corresponds to the fluorobenzyl cation (or fluorotropylium cation) resulting from cleavage of the methylene linkage between the indazole moiety and the fluorobenzyl group of the *m/z* 268 ion. The two remaining low mass ions at *m/z* 83 and 43 are the fluorocyclopentadienyl cation formed by the loss of an acetylene from the *m/z* 109 ion²⁰ and the acetyl cation formed by the loss of the 1-(2-, 3-, or 4-fluorobenzyl)-1*H*-indazole radical from the *m/z* 268 ion, respectively. *p*-FUBINAE was clearly differentiated from *o*- and *m*-FUBINAE in respect to the different relative abundance of the ions at *m/z* 253 and 268 (Figs. 2a–c). Although the relative abundance of the *o*-FUBINAE ion at *m/z* 253 was slightly lower

than that of the *m*-FUBINAE, these spectra overall were highly similar to each other.

Product ion scan spectra of *o*-, *m*-, and *p*-FUBINAE isomers

The fragment ion peak at *m/z* 253 is common to all of the indazole-type SCs containing a fluorobenzyl group at the N-1 position and a carbonyl group at the C-3 position. The product ion scan spectra of the *o*-, *m*-, and *p*-FUBINAE isomers for the precursor ion at *m/z* 253 were measured. Collision energy (CE) was set at 5–40 eV in increments of 5 eV. The detected product ions were identical among the three isomers at all the CEs observed. However, the three isomers differed in the relative abundances of the product ion at *m/z* 109 and the precursor ion at *m/z* 253 at the CEs ranging from 5 to 15 eV (Figs. 4a–c). The relative abundances of the *p*-FUBINAE ion at *m/z* 109 at the CEs of 5 and 10 eV were higher than those of the *o*- and *m*-FUBINAE, while the relative abundance of the *o*-FUBINAE ion at *m/z* 109 at 10 eV was slightly higher than that of *m*-FUBINAE (Figs. 4a and 4b). The relative abundance of the *m*-FUBINAE ion at *m/z* 253 at 15 eV was higher than that of the *o*- and *p*-FUBINAE, while that of the *o*-FUBINAE ion at *m/z* 253 was slightly higher than for *p*-FUBINAE (Fig. 4c). In the product ion scan

Fig. 4 Product ion scan spectra of *o*-, *m*-, and *p*-FUBINAE at the collision energies of (a) 5 eV, (b) 10 eV, and (c) 15 eV for the precursor ion at m/z 253.

spectra in the range of 20–40 eV, no significant differences among the three isomers were found. **Breakdown curves of *o*-, *m*-, and *p*-FUBINAE isomers**

In order to further clarify the behaviors of the ions at m/z 109 and 253, the abundance of a selected ion, expressed as a percentage of the total ion current (%TIC), was plotted against CE to yield a breakdown curve (Figs. 5a–c). CE was varied from 4 to 16 eV (4–10 eV in increments of 1 eV and 10–16 eV in increments of 2 eV). The abundances of the selected ions were presented as the areas of the mass chromatographic peaks in order to avoid the effect of spectral skewing. Individual data were fitted using the Boltzmann sigmoidal function with nonlinear regression analysis²¹. The fragment ion at m/z 109 was mainly produced by a CID reaction. Only small amounts of the product ions at m/z 205, 224, 225, and 235 were observed, and there were little differences in the total yields of their ions at any given CE. This data suggested that the decomposition of the precursor m/z 253 ion almost always produced

the m/z 109 ion. Although the patterns of the breakdown curves were similar, the sigmoidal-shaped growth (m/z 109) and decay (m/z 253) curves intersected at different CE values for each of the three isomers (*para* < *ortho* < *meta*), indicating that the three isomers required different energies to cleave the fluorobenzyl group from the indazole moiety. The two ions at m/z 109 and 253 showed very small variabilities attributed to good repeatability of mass spectral analysis. The standard deviations determined by the variabilities of the %TIC abundances of m/z 109 and m/z 253 were <1.5 and <1.7, respectively ($n=5$). The logarithmic values of the abundance ratios of the ion at m/z 109 to m/z 253 ($\ln(A_{109}/A_{253})$) were plotted against the CE in the range of 4–10 eV (Fig. 6). The $\ln(A_{109}/A_{253})$ values increased in the order of *meta* < *ortho* < *para*. In addition, the $\ln(A_{109}/A_{253})$ values for each isomer increased linearly relative to CE with high correlation coefficients, decisively separating the three approximation lines. In order to assure the significances between the $\ln(A_{109}/A_{253})$ values of

Fig. 5 Breakdown curves of the ions at m/z 109, 205, 224, 225, 235, and 253 for (a) *o*-FUBINAE, (b) *m*-FUBINAE, and (c) *p*-FUBINAE for the precursor ion at m/z 253. Error bars to the plots of m/z 109 and 253 represent standard errors ($n = 5$).

the three isomers, analysis of variance (ANOVA) followed by multiple pairwise comparisons as post hoc analysis was carried out. Bartlett's test for the homogeneity of variances showed that the $\ln(A_{109}/A_{253})$ values at the CEs of 4, 5, 6, 8, 9 and 10 eV were normally distributed (parametric) and the values at 7 eV were nonparametric. For the parametric data, one-way ANOVA followed by

Fig. 6 The plots of the logarithmic values of the abundance ratios of the ion at m/z 109 to m/z 253 ($\ln(A_{109}/A_{253})$) at the collision energies ranging from 4 to 10 eV for *o*-, *m*-, and *p*-FUBINAE. Error bars represent standard errors ($n = 5$).

Table 1 The results of analysis of variance (ANOVA) and multiple pairwise comparisons for the logarithmic values of the abundance ratios of the product ion at m/z 109 to the precursor ion at m/z 253 of *o*-, *m*-, and *p*-FUBINAE.

Collision energy (eV)	ANOVA ^a (α)	Multiple pairwise comparisons ^b (p)		
		<i>ortho</i> $\times$ <i>meta</i>	<i>ortho</i> $\times$ <i>para</i>	<i>meta</i> $\times$ <i>para</i>
4	2.7×10^{-14}	1.8×10^{-6}	1.7×10^{-6}	1.7×10^{-6}
5	5.1×10^{-16}	1.7×10^{-6}	1.7×10^{-6}	1.7×10^{-6}
6	3.4×10^{-11}	1.4×10^{-5}	1.7×10^{-6}	1.7×10^{-6}
7	2.5×10^{-3}	4.3×10^{-2}	2.5×10^{-2}	2.5×10^{-2}
8	8.4×10^{-16}	1.7×10^{-6}	1.7×10^{-6}	1.7×10^{-6}
9	2.2×10^{-13}	2.0×10^{-6}	1.7×10^{-6}	1.7×10^{-6}
10	9.2×10^{-13}	2.1×10^{-6}	1.7×10^{-6}	1.7×10^{-6}

^a one-way ANOVA (4, 5, 6, 8, 9, and 10 eV), Kruskal-Wallis test (7 eV)

^b Tukey's test (4, 5, 6, 8, 9, and 10 eV), Steel-Dwass test (7 eV)

multiple pairwise comparisons of Tukey's test was carried out. The $\ln(A_{109}/A_{253})$ values of the three isomers were shown to be significantly different at each CE ($\alpha < 3.4 \times 10^{-11}$) (Table 1). Tukey's test revealed low p values at each CE ($p < 1.4 \times 10^{-5}$). For the nonparametric data at the CE of 7 eV, the Kruskal-Wallis test and multiple pairwise comparisons using the Steel-Dwass test also showed that three isomers were significantly different ($\alpha = 2.5 \times 10^{-3}$, $p < 2.5 \times 10^{-2}$). These results showed that the differences in the $\ln(A_{109}/A_{253})$ values of the three isomers at the CEs of 4–10 eV were statistically significant.

Comparisons of synthetic cannabinoids with *o*-, *m*-, and *p*-FUBINAE

The product ion scan analyses for both the FUBINAE positional isomers and the AB-FUBINACA isomers were carried out under the same instrumental conditions. Comparing the $\ln(A_{109}/A_{253})$ plots of the FUBINAE isomers with those of the AB-FUBINACA isomers revealed that the AB-FUBINACA isomers behaved just like their corresponding positional FUBINAE isomers (Figs. 7a–c). This suggested that the relative abundances of the ions at m/z 109 and 253 in the product ion scan spectra were nearly independent of the structures of the analytes themselves, because the given energies of the CID reaction were sufficiently higher than the internal energies²²⁾ of the precursor m/z 253

ion. The $\ln(A_{109}/A_{253})$ plots of ADB-FUBINACA, FUB-AMB, FUB-APINACA, FUB-NPB-22, and FU-PX-2, all of which contain a *p*-fluorobenzyl group, also corresponded to that of *p*-FUBINAE (Figs. 8a–e). Likewise, the plot of the methanol extract from the herbal product laced with AB-FUBINACA also corresponded with the plot of *p*-FUBINAE (Fig. 8f). All these results indicated that the fluorine substitution position on the phenyl ring of indazole-type SCs containing a fluorobenzyl group at the N-1 position and a carbonyl group at the C-3 position can be elucidated by collating the $\ln(A_{109}/A_{253})$ plots of *o*-, *m*-, and *p*-FUBINAE. The verification of our present method is now being further investigated, especially between *o*- and *m*-SCs.

Conclusions

We synthesized three model compounds of indazole-type SCs containing a fluorobenzyl group at the N-1 position and a carbonyl group at the C-3 position, namely *o*-, *m*-, and *p*-FUBINAE. Although their full scan and product ion scan mass spectra were very similar to each other, they were differentiated by plotting the logarithmic values of the abundance ratios of the product ion at m/z 109 to the precursor ion at m/z 253 against collision energy. Comparing

Fig. 7 Comparisons of the $\ln(A_{109}/A_{253})$ plots of (a) AB-FUBINACA with *p*-FUBINAE, (b) AB-FUBINACA *o*-fluorobenzyl isomer with *o*-FUBINAE, and (c) AB-FUBINACA *m*-fluorobenzyl isomer with *m*-FUBINAE.

Fig. 8 Comparisons of the $\ln(A_{109}/A_{253})$ plots of (a) ADB-FUBINACA, (b) FUB-AMB, (c) FUB-APINACA, (d) FUB-NPB-22, (e) FU-PX-2, and (f) methanol extract from herbal blend containing AB-FUBINACA with a set of *o*-, *m*- and *p*-FUBINAE.

each of the plots of *o*-, *m*-, and *p*-FUBINAE with the three AB-FUBINACA positional isomers under the same instrumental conditions, the plots of the AB-FUBINACA isomers corresponded with those of the FUBINAE isomers containing the same fluorine substitution position on the phenyl ring. In addition, the plots of several SCs with a *p*-fluorobenzyl group (ADB-FUBINACA, FUB-AMB, FUB-APINACA, FUB-NPB-22, and FU-PX-2) also corresponded with that of *p*-FUBINAE, as did the methanol extract of the herbal product containing AB-FUBINACA. Therefore, it was concluded that the fluorine substitution position of indazole-type SCs containing a fluorobenzyl group at the N-1 position and a carbonyl group at the C-3 position can be clarified by collating the data set of *o*-, *m*-, and *p*-FUBINAE. Our

methodology should also be useful for narrowing down reference standard candidates of the positional isomers of an analyte. We believe that the presented differentiation method will become imperative for the forensic analysis of designer drugs.

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