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Application News

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Drugs Analysis / GCMS-TQ8050

Detection and Differentiation of Positional and Structural Isomers of Synthetic Cannabinoids Using Gas Chromatography Product Ion Spectrometry

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❑ Introduction

Forensic chemists face the challenge of identifying new psychoactive substances (NPS), whereby the general structures of controlled drugs are modified to evade legislative banning. Hence, a plethora of synthetic cathinones and cannabinoids has been available to the public, causing a global social problem. It is therefore crucial that analytical methods are developed for identifying and differentiating new analogues of synthetic drug substances, to prevent potential trading and abuse. Additionally, drug legislation is made more challenging with the use of synthetic cannabinoid containing products, typically marketed as “herbal mixtures”, which consists of analogues of synthetic cannabinoids. In this study, simultaneous multi-reaction monitoring (MRM) and product ion scan (PIS) acquisition methods have been developed for detection and differentiation of isomeric synthetic cannabinoids.

❑ Experimental

Methods and Materials

Data were acquired using a GC-TQMS system (GCMS-TQ8050, Shimadzu Corporation, Japan) in electron ionization mode. Separation was achieved using a SH-Rxi-5Sil MS capillary column (30 m × 0.25 mm × 0.25 µm). Two classes of synthetic cannabinoids were analysed. They are a mixture of JWH-018 (1-pentyl-3-(1-naphthoyl)indole) and its seven structural isomers (Figure 2), and a mixture of four positional isomers of fluoro-PB-22 (Quinolin-8-yl 1-pentyfluoro-1H-indole-3-8-carboxylate) (Figure 4). 10 µg/ml synthetic cannabinoids standards were used for method development and stability tests.

Figure 1. GCMS-TQ8050

❑ Results and Discussion

A. Method Development for JWH-018 Isomers

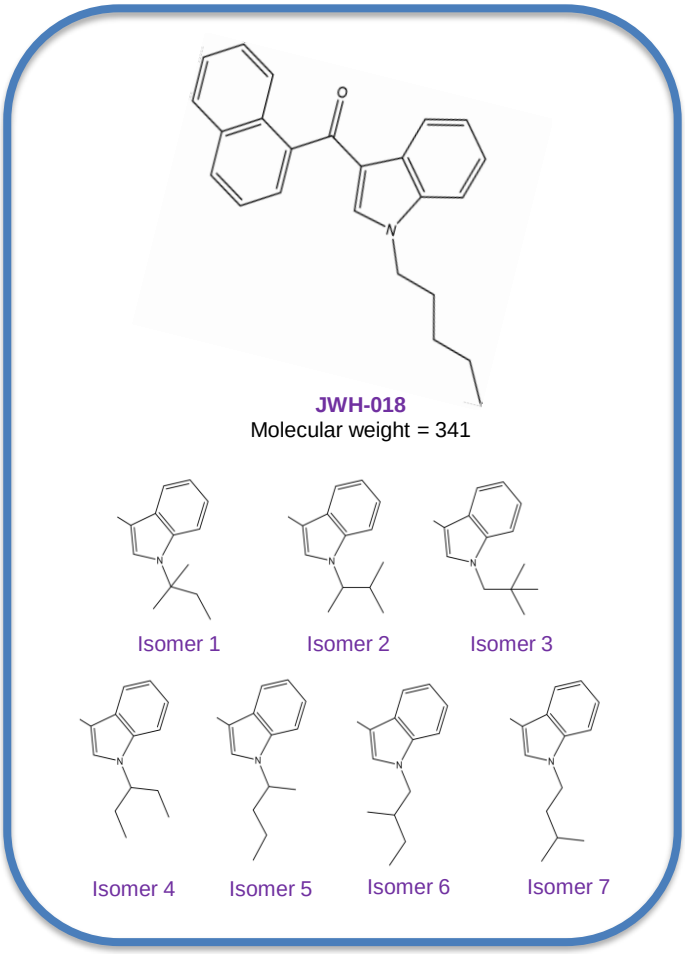


Figure 2. Molecular structure of JWH-018 and sub-structures of isomers

A-1. Identification Points of JWH-018 Isomers

JWH-018 and its seven structural isomers were identified and differentiated using the identification points below:

1. **1 target MRM transition**
2. **2 reference MRM transitions**
   - MRMs should consist of a distinctive precursor and/or product ion
3. **Product Ion Scan (PIS) mass spectra**
   - PIS was acquired from m/z 341 at an optimized single collision energy (10V)
   - Mass spectra should consist of 1 target ion and 1 or more reference ion(s), and the ion ratio% of the reference ions should fall within the tolerance range of the set ion ratio%
   - Similarity to PIS mass spectrum registered be ≥ 80% (i.e. similarity index% ≥ 80)

Table 1. MRM transitions of JWH-018 structural isomers

No.	Target	Target MRM	Ref MRM (1)	Ref MRM (2)
1	JWH-018	341 >284	341 >324	284 >167
2	Isomer 1	341 >254	341 >312	312 >155
3	Isomer 2	298 >155	341 >298	298 >170
4	Isomer 3	341 >284	284 >155	284 >167
5	Isomer 4	341 >312	312 >155	312 >254
6	Isomer 5	341 >298	298 >155	298 >170
7	Isomer 6	284 >167	284 >155	341 >284
8	Isomer 7	341 >284	284 >167	284 >254

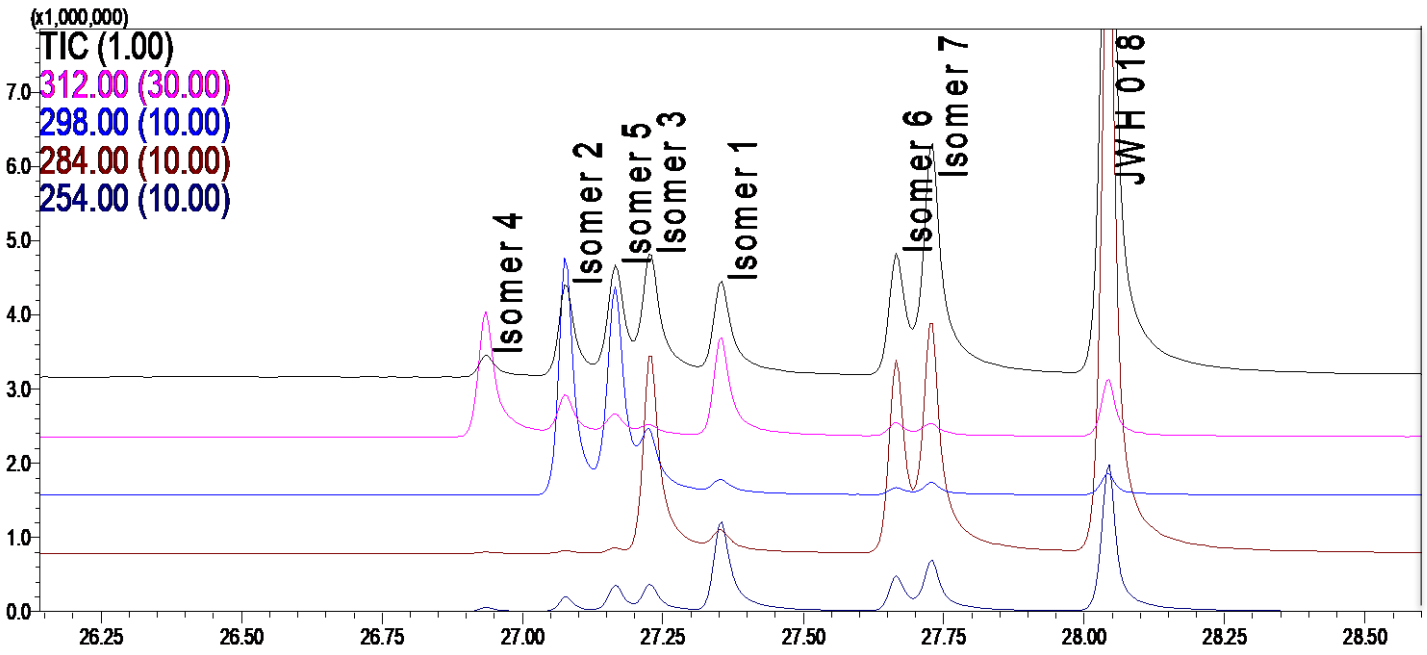
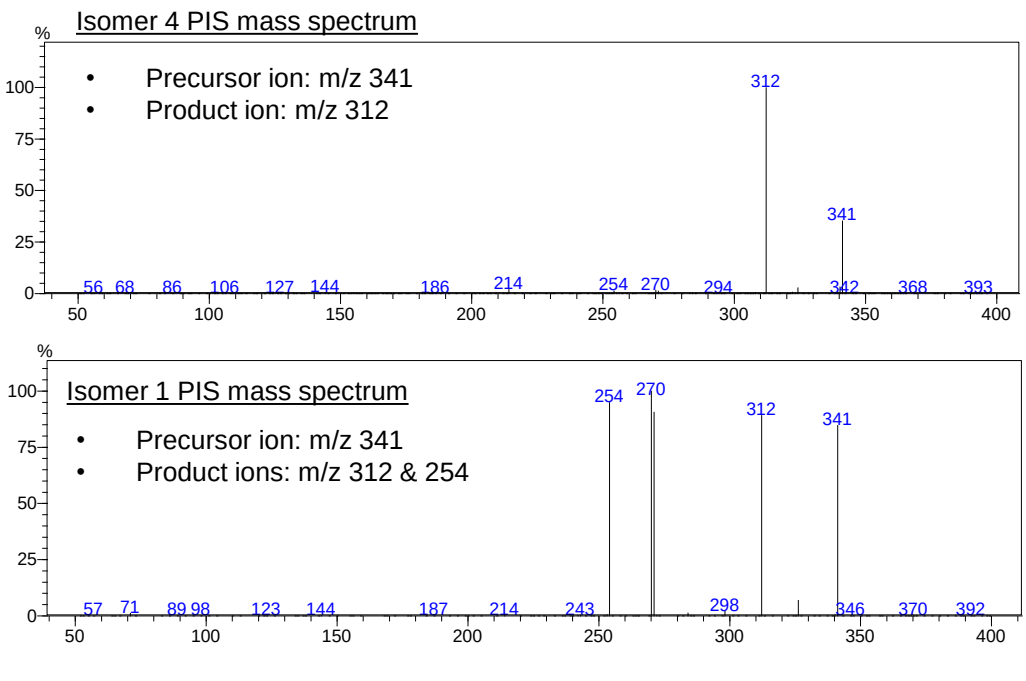
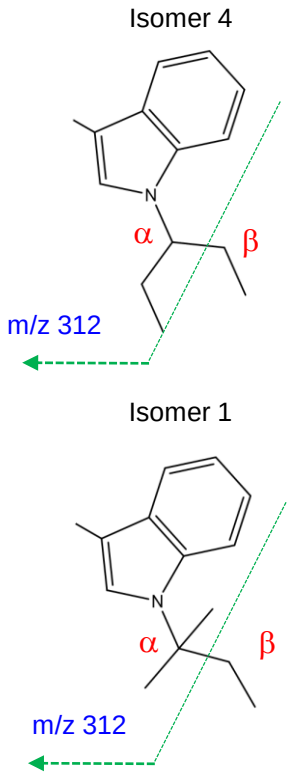


Figure 3. TIC profile of JWH-018 & isomers

E.g. Differentiation of isomers 4 & 1 via PIS





After the C_a-C_b bond cleavage, stability of the resulting carbocation resulted in different product ions, i.e. distinctive PIS mass spectra.

B. Method Development for Fluoro-PB-22 Isomers

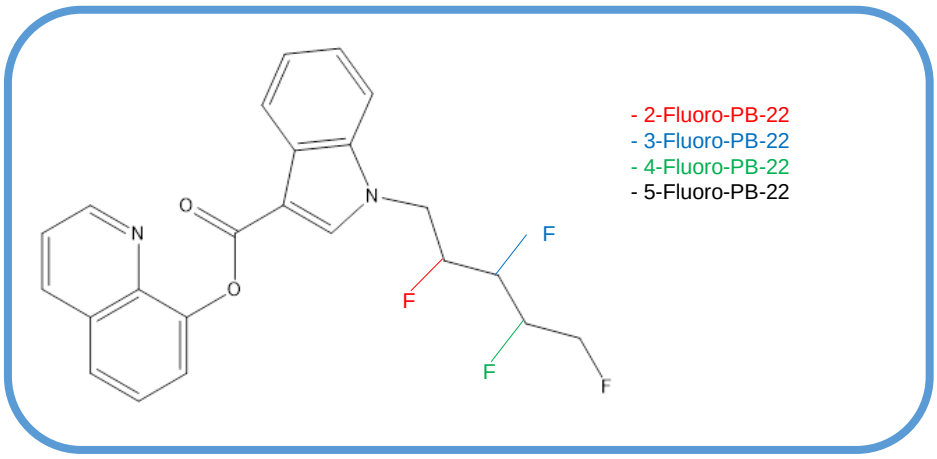


Figure 4. Molecular structures of Fluoro-PB-22 positional isomers

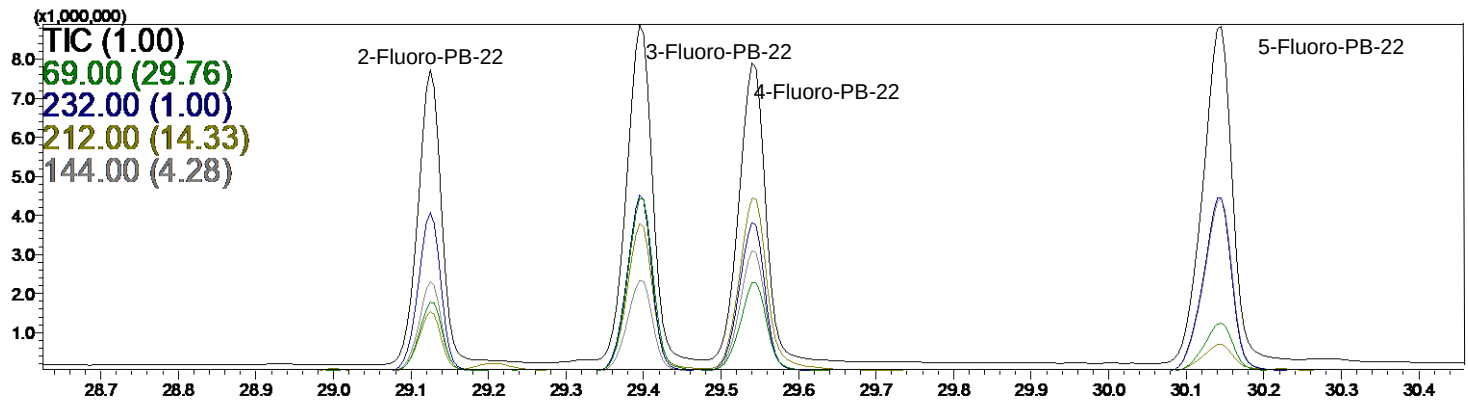


Figure 5. TIC profile of Fluoro-PB-22 positional isomers

B-1. Identification Points of Fluoro-PB-22 Isomers

Fluoro-PB-22 positional isomers were identified and differentiated using the identification points below:

1. **1 target MRM transition**
2. **2 reference MRM transitions**
3. **Product Ion Scan (PIS) mass spectra**
   - PIS was acquired from m/z 232 at an optimized single collision energy (14V)
   - Mass spectra should consist of 1 target ion and 1 or more reference ion(s), and the ion ratio% of the reference ions should fall within the tolerance range of the set ion ratio%
   - Similarity to PIS mass spectrum registered be ≥ 80% (i.e. similarity index% ≥ 80)

Table 2. MRM transitions of Fluoro-PB-22 positional isomers

Target MRM	Ref MRM (1)	Ref MRM (2)
232>144	144>116	116>89

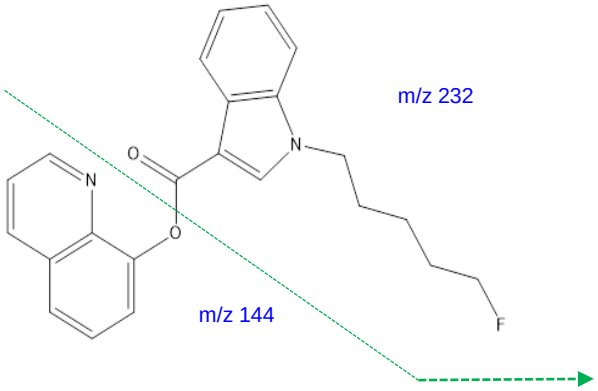
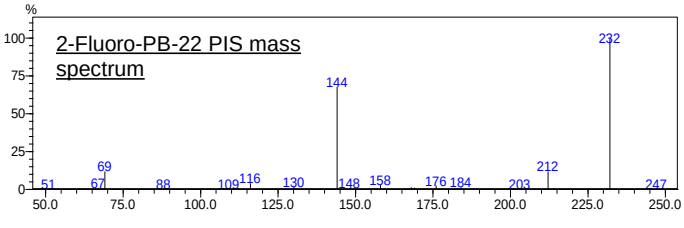


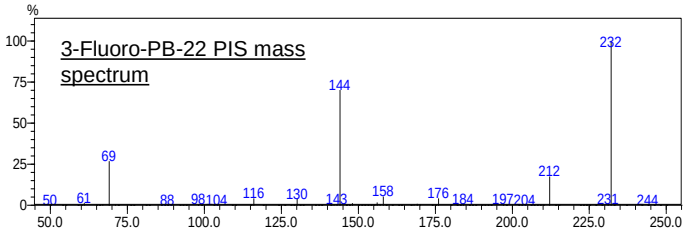
Table 3. PIS monitoring ions of Fluoro-PB-22 positional isomers

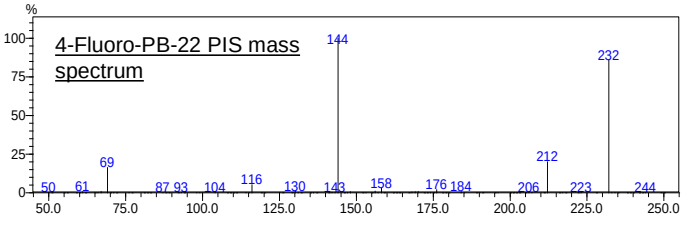
		2-Fluoro-PB-22	3-Fluoro-PB-22	4-Fluoro-PB-22	5-Fluoro-PB-22
Type	**m/z**	**Set ion ratio% (area count relative to target ion)**			
Target	232	100	100	100	100
Ref 1	144	80.00	81.17	72.73	58.66
Ref 2	69	14.80	31.05	16.93	8.13
Ref 3	212	12.01	16.73	18.30	1.60

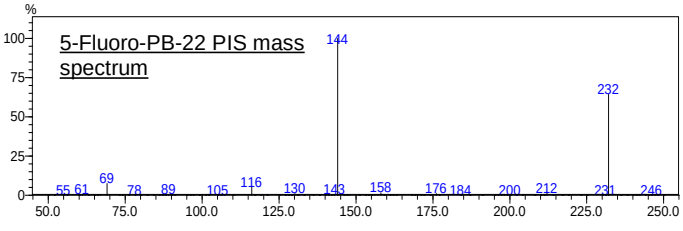
Unlike the JWH-018 isomers, the Fluoro-PB-22 isomers did not produce distinctive product ions after ionization. Therefore the %RSD of monitoring ions (from the PIS mass spectra) is crucial to method reliability. Details of monitoring ions are listed in Table 3.

Differentiation of Fluoro-PB-22 positional isomers via PIS









B-2. Method Validation

To ensure method stability and reliability, intra- and inter-day stability tests were done for a period of 3 consecutive days. The %RSD of PIS monitoring ions and similarity index% were monitored. In a day, 7 replicate analyses of 10 µg/ml standards were performed for each set of isomers. Each set of isomers was analysed separately.

For JWH-018 isomers, the highest intra-day %RSD of monitoring ions ratio% and similarity index were 5.38% and 0.80%, respectively. The highest inter-day %RSD of monitoring ions ratio% and similarity index were 1.78% and 0.41%, respectively. For Fluoro-PB-22 isomers, the highest intra-day %RSD of monitoring ions ratio% and similarity index were 5.55% and 0.80%, respectively. The highest inter-day %RSD of monitoring ions ratio% and similarity index were 0.68% and 0.22%, respectively.

C. Method Application

Subsequently, the MRM/PIS methods of each set of isomers were combined into a single acquisition method. In this way, samples which may contain mixtures of synthetic cannabinoids can be analysed efficiently within a single run. With high similarity match to standard PIS mass spectra, JWH-018 and 5-Fluoro-PB-22 were detected (Figures 6 & 7).

❑ Conclusion

Comprehensive detection and differentiation of structural and positional isomers of JWH-018 and Fluoro-PB-22, respectively, was achieved using simultaneous MRM and PIS with GC-TQMS. The method developed was tested to be stable and reliable for real NPS samples.

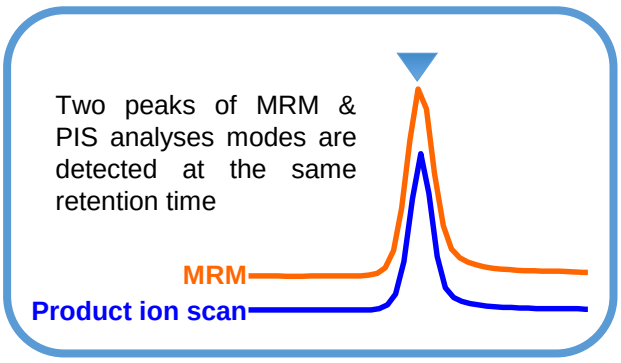
ID#	Name	SI	Ret.Time
1	JWH 018 N-(1-ethylpropyl) isomer; Isomer4	No peak is detected.	
2	Isomer 4 PIS	Ratio of reference ion does not match.	
3	JWH 018 N-(1,2-dimethylpropyl) isomer; Isomer2	No peak is detected.	
4	Isomer 2 PIS	Ratio of reference ion does not match.	
5	JWH 018 N-(1-methylbutyl) isomer; Isomer5	No peak is detected.	
6	Isomer 5 PIS	Ratio of reference ion does not match.	
7	JWH 018 N-(2,2-dimethylpropyl) isomer; Isomer3	No peak is detected.	
8	Isomer 3 PIS	Ratio of reference ion does not match.	
9	JWH 018 N-(1,1-dimethylpropyl) isomer; Isomer1	No peak is detected.	
10	Isomer 1 PIS		not match.
11	JWH 018 N-(2-m		w/Band range.
12	Isomer 6 PIS		not match.
13	JWH 018 N-(3-methylbutyl) isomer; Isomer7	No peak is found in Window/Band range.	
14	Isomer 7 PIS	Ratio of reference ion does not match.	
15	JWH 018	68	27.403
16	JWH018 PIS	99	27.399

Positive detection of JWH018 in sample A

Figure 6. Positive detection of JWH-018 with 99% PIS mass spectrum similarity match to standard PIS mass spectrum.

17	2-Fluoro-PB-22	Ratio of reference ion does not match.	
18	2-Fluoro-PB-22 PIS	No peak is found in Window/Band range.	
19	3-Fluoro-PB-22 PIS	No peak is found in Window/Band range.	
20	3-Fluoro-PB-22	Ratio of reference ion does not match.	
21	4-Fluoro-PB-22 PIS	No peak is found in Window/Band range.	
22	4-Fluoro-PB-22	Ratio of reference ion does not match.	
23	5-Fluoro-PB-22 PIS	97	29.304
24	5-Fluoro-PB-22	69	29.306

Figure 7. Positive detection of 5-Fluoro-PB-22 with 97% PIS mass spectrum similarity match to standard PIS mass spectrum.



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