# Analysis of Synthetic Cannabinoids in Herbal Products

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**ABSTRACT**: "Legal highs" present as synthetic cannabinoids in herbal products and started to appear and marketed as "*spice*" via internet websites and specialized headshops in 2004. These products are often advertised as producing cannabis effects when smoked. Recently, several synthetic cannabinoids that produce cannabis-like effect had been identified as adulterants in herbal products. It is anticipated that synthetic cannabinoids will be controlled in Malaysia in near future. In this study, gas chromatography-mass spectrometry (GC-MS) was used to identify synthetic cannabinoids in herbal products while liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify the compound present in herbal products using mixed standard solutions (JWH-018, JWH-073, and CP-47,497). To study of the homogeneity of compound in herbal products, herbal products were divided into 20 sampling points with equal size (12 cm x 12 cm) without prior homogenization, where each sampling point was quantified using LC-MS/MS to investigate the variation of synthetic cannabinoids across batches of samples. Three synthetic cannabinoids JWH-018, JWH-250 and AM-2201 were identified by GC-MS analysis. LC-MS/MS results show the purity of JWH-018 was in the range 0.0431%-0.1833% for all 20 sampling points. JWH-073 was identified using LC-MS/MS at trace level (0.007 µg/mL - 0.008 µg/mL) although it was not detected by GC-MS. Our study shows that the purity of JWH-018 varied at all sampling points. As a conclusion, due to inhomogeneous distribution synthetic compounds, composite sample from sufficient sampling points is recommended in future narcotic caseworks in order to get the average content of compound present in herbal products if homogenization of the bulk samples is impossible.

**Keywords:** forensic chemistry, synthetic cannabinoids, legal high, JWH-018

## Introduction

Over the past decade, an increasing number of so-called "legal highs" substances have been abused as recreational drugs especially by young people in United State (US), Europe and Australia (Johnson *et al.*, 2013). "Legal highs" are substances with psychotropic action that are purposely marketed for recreational use by exploiting inadequacy of existing control substances legislation [1]. Examples of "legal highs" products are *Spice* [2-4] and *Bath Salts* marketed via internet or in specialized shops around 2004 [5]. European Monitoring Centre for Drug and Drug Addiction (EMCDDA) and Europol found that the *Spice* smoking mixture was not the herbal products that it purported to be, rather, it contained several synthetic cannabinoids as adulterants in the herbal smoking products [6]. Synthetic cannabinoids are referred to compounds with similar chemical structures and substances with structural features which allow them to bind to one of the known cannabinoid receptors ($CB_1$ and $CB_2$) [7]. Although they are commonly called as synthetic cannabinoids, many of the compound are not structurally similar and related to the naturally cannabinoids such as $\Delta^9$-THC, Figure 1.

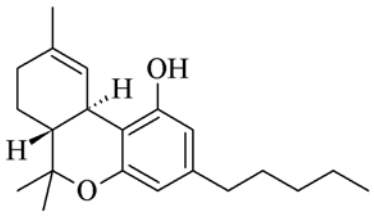
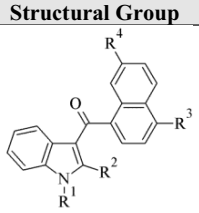
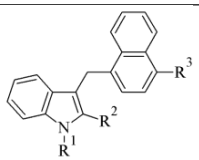
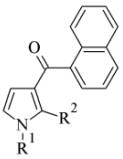
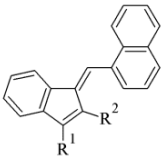
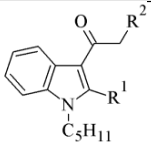
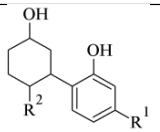
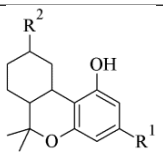
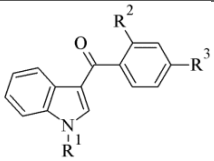



Figure 1: Molecular structure of $\Delta^9$-tetrahydrocannabinol (THC) [8]

Synthetic cannabinoids could be subdivided into 'classical cannabinoids' which are structurally similar to $\Delta^9$-THC such as HU-210, and 'non-classical cannabinoids' such as

'CP-47,497' which are structurally unrelated to $\Delta^9$-THC [6]. Most synthetic cannabinoids consist of 22 to 26 carbon atoms, non-polar and are lipid soluble [8]. Therefore synthetic cannabinoids would be expected to volatilise when smoked. Generally, they can be classified into one of the eight major structural groups [9-10], Table 1.

Table 1: Structural groups and examples of their respective examples (Source: ACMD, 2009 and 2012, [9-10])

| Cannabinoids | Structural Group | Examples |
|---|---|---|
| Naphthoylindoles |  | JWH-015 $R^1$=propyl $R^2$=methyl<br>JWH-018 $R^1$=pentyl<br>JWH-019 $R^1$=hexyl<br>JWH-073 $R^1$=butyl<br>JWH-081 $R^1$=pentyl $R^3$=methoxy<br>JWH-200 $R^1$=morpholinylethyl<br>JWH-210 $R^1$=pentyl $R^3$=ethy |
| Naphthylmethylindoles |  | JWH-184 $R^1$=pentyl $R^3$=methyl<br>JWH-185 $R^1$=pentyl $R^3$=methoxy |
| Naphthoylpyrroles |  | JWH-145 $R^1$=pentyl $R^2$=phenyl<br>JWH-150 $R^1$=butyl $R^2$=phenyl |
| Naphthylmethylindenes |  | JWH-176 $R^1$=pentyl |
| Phenylacetylindoles |  | JWH-250 $R^2$=2-methoxyphenyl<br>JWH-253 $R^1$=methyl<br>$R^2$=3-methoxyphenyl<br>JWH-311 $R^2$=2-fluorophenyl |
| Cyclohexylphenols |  | CP-47,497 $R^1$=1,1-dimethylheptyl<br>CP-55,940 $R^1$=1,1-dimethylheptyl,<br>$R^2$= hydroxypropyl |
| Classical Cannabinoid |  | HU-210 $R^1$=1,1-dimethylheptyl<br>$R^2$=hydroxymethyl<br><br>Nabilone $R^1$=1,1-dimethylheptyl<br>$R^2$=keto (=O) |
| Benzoylindoles |  | AM-694 $R^1$=5-fluoropentyl<br>$R^2$=I<br><br>RCS-4 $R^1$=5-fluoropentyl<br>$R^3$=methoxy |

(Note: *R= H if not specified)

At present, the actual production of the herbal products with synthetic cannabinoids such as *Spice* remained unclear. It is assumed that the production was started by dissolving the compounds in a solvent, then spray the solution on the plant materials, before being evaporate to dryness and packaged as herbal mixtures. As a result, the psychotropic effects of these herbal mixtures are actually from synthetic cannabinoids deposited on the surface of plant material, rather than from the natural components of the original plant materials. Synthetic cannabinoids that have been frequently detected in *spice* in Europe are JWH-018, JWH-073, JWH-398, JWH-250, HU-210, CP-47,497 and its homologues, and oleamide [11].

It is anticipated that synthetic cannabinoids will be put under control by Malaysia law in near future. The methodology used for its analysis must consider the homogeneity of samples and to satisfy the requirement in various analytical analyses. We studied the use of GC-MS and LC-MS-MS for the detection of cannabinoids and investigated the homogeneity of herbal products submitted for analysis [12-16].

**Materials and methods**

HPLC grade methanol and pure reference materials JWH-018, JWH-073 and CP-47,497 were obtained from LabChem (Malaysia). A seized bulk sample of herbal products was obtained from Narcotic Section, Department of Chemistry, Malaysia.

***Gas chromatography-mass spectrometry (GC-MS) analysis***

Approximately 2 g of the herbal product was placed in a 50 mL beaker and 10 mL of methanol was added. The mixture was sonicated for 10 min and allowed to stand for 10 min at room temperature. The methanol extract was then isolated for GC-MS analysis using an Agilent GCMS 6890N equipped with an Agilent HP-5 ms capillary column (30 m length, 0.25 µm i.d., and 0.25 µm film thicknesses). The injector was operated in split mode (70:1) at 200°C. Helium gas was used as the carrier gas at a flow rate of 0.7 mL/min. The oven temperature was held at 80°C for 1 min and ramped to 310°C at a rate of 10 °C/min and held for 5 min. The injection volume was 1.0 µL using a 10 µL syringe. The Agilent 5975B MS detector was operated at 280°C. Data was collected at scan mode from 50-500 m/z and analysed using Chemstation software.

***Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis***

*Sampling points preparation for LC-MS/MS*

The sampling points were prepared by a modified "cone and quartering" method. A seized sample of herbal products was flattened into a rectangular shape on paper by a 30 cm ruler. The herbal products were divided equally into 20 sampling point. The size of each sampling point was approximately 12 cm x 12 cm, giving a total of 20 sampling points, with each square sampling point being labeled with a number, from 1 to 20 (Figure 2).

Figure 2: Preparation of square sampling points 1 to 20

Two set of samples were taken by random sampling from each sampling point. The first set of sample was labeled as "a" and the second set was labeled as "b". Approximately 100 mg of herbal products from one sampling point was placed in a 10 mL volumetric flask and 10 mL of methanol was added. The volumetric flask was shaken slightly to make it homogenous. This was sonicated for 5 min and then left for 10 min to settle. The methanol extract was filtered using 0.2 µm syringe and transferred into 2 mL vial. The filter extracts were used for LC-MS/MS analysis. Set b was then taken from locations other than those taken by samples in set a.

LC-MS/MS analysis was carried out with a Water Alliance 2695 Liquid Chromatography (LC) separation module coupled with Waters Micromass Quattro Premier Tandem Quadrupole Mass Spectrometer (MS/MS). The chromatographic separation was performed using an Onyx Monolithic C18 column (50 mm X 2.0 mm, Phenomenex). The column temperature was maintained at 30°C with mobile phase flow rate of 0.3 mL/min.

The mobile phase A was 0.1% formic acid in water whereas the mobile phase B was 0.1% formic acid in acetonitrile. The gradient elution was started with 50% of mobile phase B and increased to 80% of mobile phase B within the first 5 min. The 80% of mobile phase B was held for 3 min (isocratic). Then, starting conditions were restored within 2 min, and kept for 2 min for column equilibration. The sample injected volume was 10 µL.
Quattro Premier Tandem Quarupole MS (Waters) with electrospray positive ionization (ESI+) mode has the following setting: Capillary voltage 3.0 kV, source temperature of 130°C, desolvation temperature of 350°C, desolvation gas flow rate of 650 L/hr with nitrogen, cone gas flow rate of 20 L/hr with nitrogen, cone voltage 30 V and an argon gas pressure of 3.6 X $10^{-3}$ mbar (used as collision energy). Synthetic cannabinoids was analysed by multiple reaction monitoring mode (MRM) as in Table 2.

Table 2: The MRM conditions of JWH-018, JWH-073, and CP-47,497

| No | Standards | Parent | Daughter | Cone Voltage (V) | Collision Energy (V) |
|---|---|---|---|---|---|
| 1 | JWH-018 | 342.05 | 154.70 | 42.00 | 22.00 |
| | | | 126.65 | 42.00 | 43.00 |
| 2 | JWH-073 | 328.15 | 154.70 | 37.00 | 25.00 |
| | | | 126.65 | 37.00 | 28.00 |
| 3 | CP-47,497 | 319.15 | 301.20 | 12.00 | 8.00 |
| | | | 233.05 | 12.00 | 15.00 |

JWH-018, JWH-073, and CP-47,497 standard were diluted with methanol to prepare the mixed standard solution of 10, 20, 50, 100 and 200 ng/mL for calibration curves. The concentration of JWH-018 (µg/mL) was converted to purity of JWH-018 (%) using formula below:

$$\text{Purity of drug (\%)} = \frac{\text{concentration } \left(\frac{\text{mg}}{\text{mL}}\right) \text{x 10 ml}}{\text{weight taken (mg)}} \text{ X 100\%}$$

The purity of JWH-018 of duplicate samples (a & b) for each sampling points were summed and averaged to represent the purity of JWH-018 of the square sampling points respectively. The variation of purity of JWH-018 was compared vertically and horizontally across the squares sampling points using Microsoft Excel ®.

**Results and discussion**

*Physical features of herbal products*

Herbal products were made up of greenish, brownish and yellowish unknown plant materials as shown in Figure 3. The plant materials consist of dry leaves, flowers, and stem with pungent odour.

Figure 3: Physical appearance of herbal products

*Gas chromatography-mass spectrometer (GC-MS)*

The herbal samples were found to contain JWH-250 eluted at 23.974 min, JWH-018 eluted at 25.816 min, and AM-2201 eluted at 26.932 min (Figure 4, Left), confirmed by their mass spectrum upon GC-MS analysis. Figure 4 (Right) shows the mass spectrum of JWH-018.

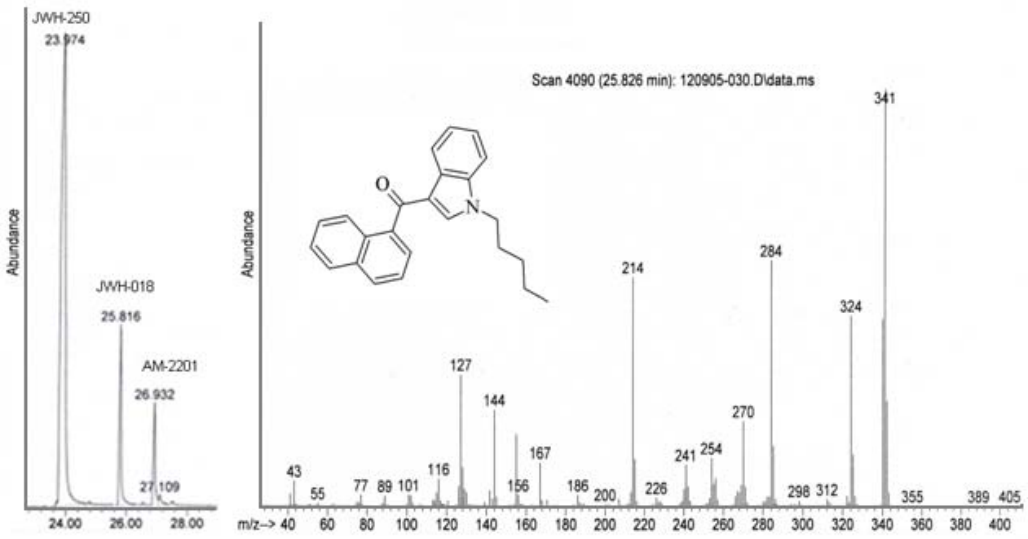



Figure 4: GC-MS chromatogram of the herbal products, three synthetic cannabinoids was identified: JWH-250 (RT=23.974 min), JWH-018 (RT=25.816 min), and AM-2201 (26.932 min), (Left) and mass spectrum of JWH-018 showing the base peak at m/z 341 and higher ion abundance at m/z 284, 214, and 324 (Right)

Although three synthetic cannabinoids were identified by GC-MS, only JWH-018 was quantitified in LC-MS-MS analysis due to the lack of the reference materials of JWH-250 and AM-2201. Note that in this study, mixed standard solutions that consist of JWH-018, JWH-073, and CP-47,497 were used. Limit of detection (LOD), limit of quantitation (LOQ), and recovery rate were preliminary determined by the laboratory. JWH-018 has LOD of 2.36 ng/mL, LOQ of 7.08 ng/mL, and recovery rate of 98.2%. JWH-073 has LOD of 1.15 ng/mL, LOQ of 3.44 ng/mL, and recovery rate of 120%. The detected concentrations (µg/mL) of JWH-018 in herbal products are converted to purity (%) of JWH-018 to compensate the variation of weight of samples taken.

The calculated purity of JWH-018 in herbal products ranged between 0.0431%-0.1833% (mean =0.1234%) with a standard deviation of 0.0443%. Table 3 shows the weight of herbal products taken, concentration of JWH-018, purity of JWH-018 and the average purity of JWH-018 for sample set a, and set b from 20 sampling points.

The line graph in Figure 5 shows that the purity of JWH-018 for sampling point 6 to 10 is lower than the line graph of other sampling points indicating the distribution of the compound of interest in the herbal products were not homogenized across the sampling points from left to right. Figure 6 shows the variation of JWH-018 concentration when sample vertically.

In this study, the difference of purity of JWH-018 between samples set a and set b from the same sampling points have also been investigated. The difference of purity of JWH-018 was ranging from 0.0071% to 0.01149% (mean= 0.0513%) indicating that the purity of JWH-018 not only varied between sampling points, but also within the same sampling points. Defensible sampling strategies and proper homogenizing of such bulk sample is therefore vital during forensic analysis [12].

Table 3: Weight of herbal product, concentration of JWH-018, purity of JWH-018, and average purity of JWH-018 for set a, and set b.

| Sampling points | Weight of herbal product (mg) | | Concentration of JWH-018 (µg/mL) | | Purity of JWH-018 (%) | | Average purity (%) |
|---|---|---|---|---|---|---|---|
| | Set a | Set b | Set a | Set b | Set a | Set b | |
| 1 | 103.37 | 104.38 | 16.027 | 22.086 | 0.1550 | 0.2116 | 0.1833 |
| 2 | 103.32 | 103.93 | 17.369 | 18.364 | 0.1681 | 0.1767 | 0.1724 |
| 3 | 106.10 | 102.10 | 16.737 | 15.378 | 0.1577 | 0.1506 | 0.1542 |
| 4 | 104.63 | 102.29 | 17.168 | 11.735 | 0.1641 | 0.1147 | 0.1394 |
| 5 | 108.94 | 101.30 | 16.155 | 9.158 | 0.1483 | 0.0904 | 0.1194 |
| 6 | 100.02 | 103.44 | 7.035 | 8.308 | 0.0703 | 0.0803 | 0.0753 |
| 7 | 103.97 | 106.22 | 5.103 | 7.549 | 0.0491 | 0.0711 | 0.0601 |
| 8 | 109.10 | 106.88 | 4.324 | 5.788 | 0.0396 | 0.0542 | 0.0469 |
| 9 | 115.43 | 107.17 | 3.432 | 6.056 | 0.0297 | 0.0565 | 0.0431 |
| 10 | 108.39 | 100.96 | 3.976 | 7.492 | 0.0367 | 0.0742 | 0.0555 |
| 11 | 109.68 | 104.11 | 6.150 | 16.625 | 0.0561 | 0.1597 | 0.1079 |
| 12 | 101.72 | 102.94 | 7.754 | 18.689 | 0.0762 | 0.1816 | 0.1289 |
| 13 | 107.42 | 103.25 | 11.440 | 17.987 | 0.1065 | 0.1742 | 0.1404 |
| 14 | 104.81 | 103.71 | 12.833 | 16.708 | 0.1224 | 0.1611 | 0.1418 |
| 15 | 109.25 | 107.22 | 13.709 | 20.352 | 0.1255 | 0.1898 | 0.1577 |
| 16 | 102.87 | 109.39 | 19.813 | 16.923 | 0.1926 | 0.1547 | 0.1737 |
| 17 | 107.74 | 100.63 | 21.024 | 13.929 | 0.1951 | 0.1384 | 0.1668 |
| 18 | 104.41 | 100.88 | 16.793 | 11.499 | 0.1608 | 0.1140 | 0.1374 |
| 19 | 105.24 | 100.90 | 19.697 | 8.934 | 0.1872 | 0.0885 | 0.1379 |
| 20 | 101.23 | 105.63 | 18.494 | 7.160 | 0.1827 | 0.0678 | 0.1253 |

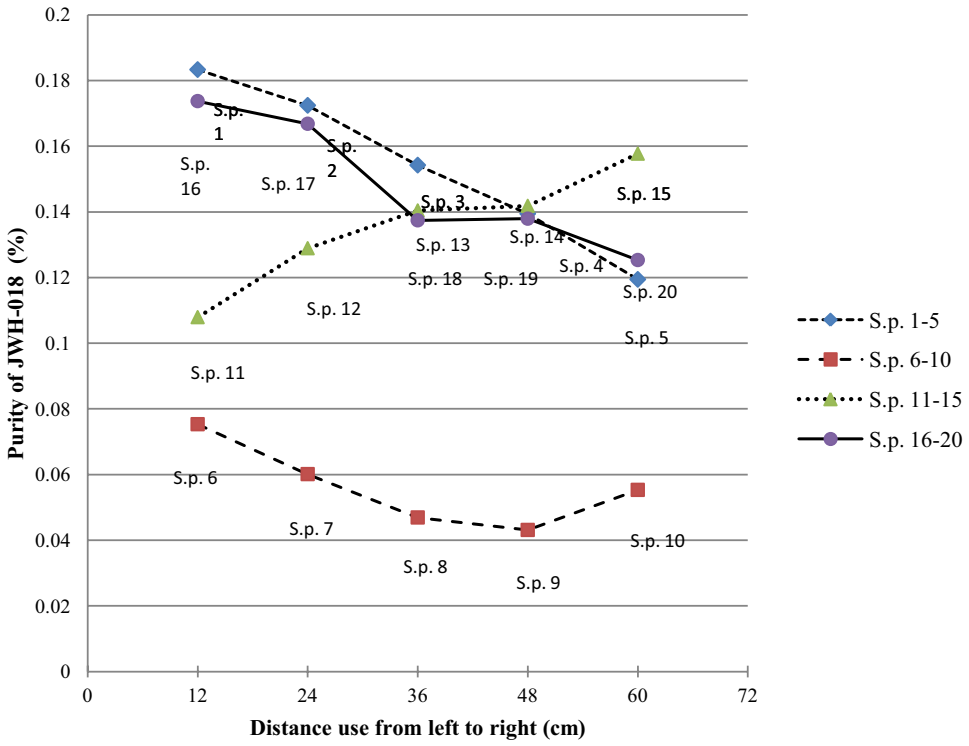



Figure 5: Variation of purity of JWH-018 in herbal products across the sampling point from left to right (horizontally) (Note: *S.p. =Sampling point).

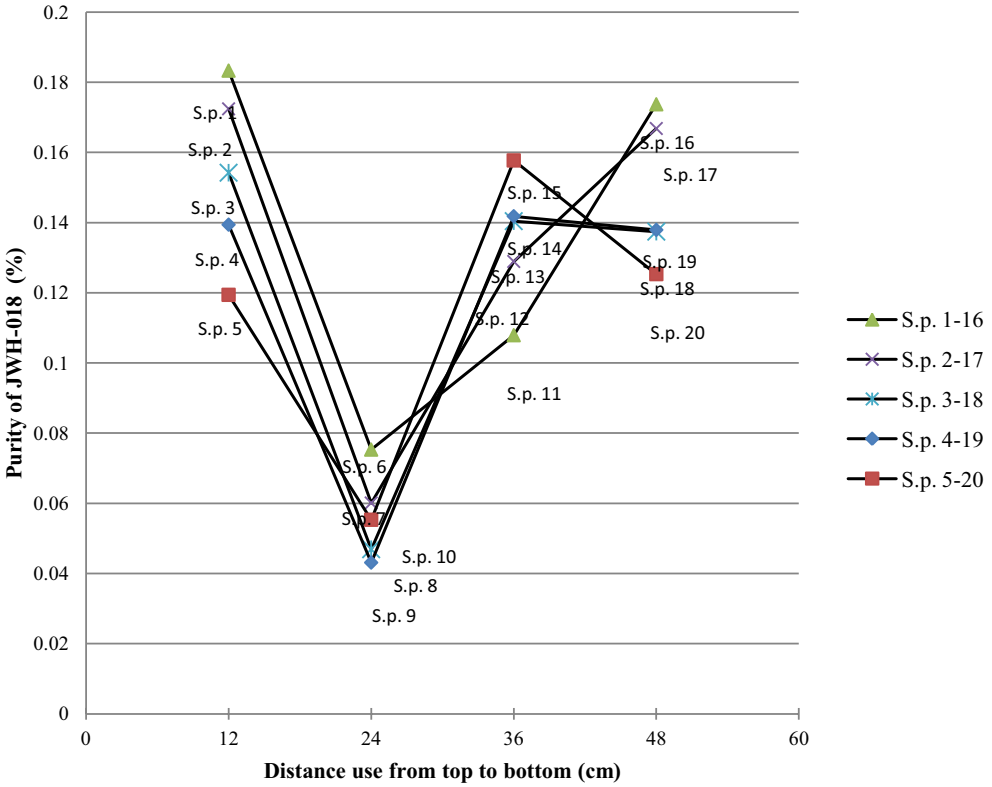



Figure 6: The variation of purity of JWH-018 in herbal products across the sampling point from top to bottom (vertically) (Note: *S.p.= Sampling point)

## Conclusion

The drug analysis in forensic narcotic casework required accuracy with little or no variation. In this study, GC-MS was used to identify the compounds and LC-MS-MS was subsequently used to quantify the purity of JWH-018 as an indicator of the purity of synthetic cannabinoids that could have present in herbal samples. Our results have indicated the purity of JWH-018 in herbal products not only varied between sampling points of 12 cm x 12 cm, but also between two locations within a sampling point. For this reason, composite samples are recommended for quantification of synthetic cannabinoids in herbal products in future forensic casework. Composite samples could give a more representative sample to present a bulk in forensic casework. To homogenize the samples, grinding the composite samples between two pieces of sandpapers is recommended as it provides better homogeneity (Choi *et al.*, 2013).

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