Thermal Degradation of Synthetic Cannabinoids Containing a Cyclopropyl Group

David Eckre, B.S.\textsuperscript{a,*}; Lee Fadness, B.S.\textsuperscript{b}; Graham Rankin, Ph.D\textsuperscript{a}; Candice Bridge, Ph.D\textsuperscript{b}

\textit{a) Marshall University, 1401 Forensic Science Drive, Huntington, WV 25701}
\textit{b) USAICIL, 4930 N 31st St, Forest Park, GA 30297}

Abstract

The newest wave of synthetic cannabinoids, e.g. UR-144 and XLR-11, contain cyclopropyl rings and therefore circumvent the new S.3187 law. The analysis of cyclopropyl containing molecules can be challenging because the chromatograms of the standards (and casework) contain multiple related peaks. Standards of UR-144 and XLR-11 were heated and then both the Unheated and the Heated samples were analyzed using GC-MS, LC-MS, solid phase GC-IR, FT-IR, Raman, and pyrolysis GC-MS. It was concluded that there were two major peaks in the chromatogram. One peak was the original molecule and the second was probably a thermodynamic product where the cyclopropyl ring was thermally opened.

1. Introduction

In 1986 the Controlled Substances Act was amended to control a general class of compounds—analogues of Schedule I and II controlled substances.\textsuperscript{1} Prior to this, only substances listed explicitly by name (and some isomers and salts of these substances) were legally controlled. Clandestine manufacturers of chemicals similar (both structurally and functionally) to controlled substances could stay one step ahead of the legal system by moving on to new analogues once the old ones had been explicitly controlled. The Controlled Substance Analogue Enforcement Act of 1986 was a counter offensive to this maneuver-- it controlled certain drugs that were structurally “substantially similar” to Schedule I or II controlled substances. The manufacturers of synthetic cannabinoids then found an ingenious way around this. They used chemicals that mimicked the effects of psychoactive substances like THC, but were not
structurally similar to them.² These substances were not controlled in any of the various countries in which they were sold.

The first wave of synthetic cannabinoids was detected in herbal smoking packages in late 2008 and included JWH-018, JWH-073, and CP-47,497. The original herbal mixtures were called ‘Spice Gold’, ‘Spice Silver’, and ‘K2’. Currently, there are many other such products all which are still collectively referred to as “Spice”.³⁻⁵ At the beginning, Spice was largely a European phenomenon, but now it is sold worldwide via ‘head’ shops or online.⁶⁻⁸ It is marketed as incense or room odorizers and declared not for human consumption.⁹,¹⁰ Spice is manufactured by spraying leafy material, e.g. Blue Lotus, Lion’s Tail, Dwarf Scullcap, and Pink Lotus, with synthetic cannabinoids. The solvent is then evaporated off, leaving the synthetic cannabinoids on the leaves.¹¹,¹² The health risks of inhaling synthetic cannabinoids are rarely tested in humans and are therefore relatively unknown.¹³ The potential therapeutic benefits of synthetic cannabinoids have not been approved for human consumption because of the undesirable psychoactive properties and potential toxic side effects.¹⁴ In 2009, JWH-018, JWH-073, and CP-47,497 were explicitly controlled in several European countries.¹⁵

A second wave of synthetic cannabinoids hit the market in 2010 and included JWH-081, AM-2201, JWH-210, and JWH-122.¹⁶ These synthetic cannabinoids were manufactured to circumvent the laws that banned the first-wave compounds. Several European countries enacted generic bans that controlled synthetic cannabinoids based on general chemical structures. For example, anything with the structure 3-(1-naphthoyl)indole was banned regardless of any substitution at the nitrogen atom of the indole ring, any further substitution on the indole ring, and any substitution on the naphthoyl ring.¹⁵
In March 2011, the DEA temporarily placed JWH-018, JWH-073, JWH-200, CP-47,497, and cannabicyclohexanol and their salts, isomers, and salts of isomers into Schedule I of the Controlled Substances Act for twelve months. The ban was later extended an additional six months. On July 9, 2012 Senate bill S.3187 was signed into law. This bill classified cannabimimetic agents’ as Schedule I controlled substances and defined them as “any substance that was a cannabinoid receptor type 1 (CB1 receptor) agonist as demonstrated by binding studies and functional assays within any of the following structural classes (Fig. 1)”. The bill also listed 16 synthetic cannabinoids by name, making them Schedule 1 controlled substances.

![Figure 1](image.png)

Figure 1. The seven general structures that are in the five classes of generically controlled structures that are now Schedule 1 drugs.

Recently, a third wave of synthetic cannabinoids have been detected in herbal smoking mixtures. These compounds were manufactured in order to circumvent the generic bans on the second-wave compounds. Some of these new synthetic cannabinoids, such as UR-144 {(1-
penty1-1H-indol-3-yl) (2,2,3,3-tetramethylcyclopropyl)methanone, Fig. 2A) and XLR-11 \{(1-
(5-fluoropentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone, Fig. 2B)\}, contain
cyclopropyl rings. The structure-activity relationships (SAR) of many synthetic cannabinoids
and how they interact with the cannabinoid receptors have been identified. The SAR of UR-
144 identified that it binds better to the CB2 receptor\textsuperscript{21} and there is currently no SAR for
XLR-11.

![Structures of (A) UR-144, (B) XLR-11, (C) XLR-11 4-Pentenyl Analog, and (D) XLR-11 4-Fluoropentyl Isomer.](image)

Many casework samples that involve compounds containing cyclopropyl rings have
multiple peaks in the chromatogram and analysts are questioning what is causing the extra peaks.
It was previously reported that two cyclopropyl ketones do thermally rearrange to homoallylic
ketones.\textsuperscript{22} It is hypothesized that the heat from smoking the synthetic cannabinoids as well as the
injection port temperature causes the cyclopropyl ring (Fig. 3A) to open, creating one of three
thermodynamic products (Fig. 3 B-C). 2,3,3-Trimethyl-1-butene (TMB, Fig. 3B) side chain
would form if the bond between carbon 1 (C1) and C3 breaks and the resulting double bond
forms between C3 and C4 2,4-Dimethyl-1-pentene (DM1P, Fig. 3C) side chain would form if the
bond between C2 and C3 breaks and the resulting double bond forms between C3 and C4. 2,4-Dimethyl-2-pentene (DM2P, Fig. 3D) side chain would form if the bond between C2 and C3 breaks and the resulting double bond forms between C1 and C3.

Figure 3. The closed cyclopropyl ring (A) can form 3 possible side chains: (B) 2,3,3-trimethyl-1-butene (TMB), (C) 2,4-dimethyl-1-pentene (DM1P), (D) 2,4-dimethyl-2-pentene (DM2P).

Standard samples of UR-144, XLR-11, XLR-11 4-Pentenyl Analog {(1-(pent-4-en-1-yl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone, Fig. 2C}, and XLR-11 4-Fluoropentyl Isomer {(1-(4-fluoropentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone, Fig. 2D} were obtained commercially. XLR-11 4-Fluoropentyl Isomer (4-FPI) and XLR-11 4-Pentenyl Analog (4-PA) were analyzed using the same conditions as UR-144 and XLR-11 to see if the additional peak in the chromatogram was caused by co-elution with either 4-PA or 4-FPI.

The term Unheated refers to the standard sample being analyzed and the term Heated refers to the heated sample being analyzed. Each of the standard samples was heated externally at various temperatures to determine if the cyclopropyl ring could be opened and if it does, at what temperature it would open. Both the Unheated and the Heated samples were analyzed by gas chromatography – mass spectroscopy (GC-MS), liquid chromatography – MS (LC-MS), solid-phase GC- infrared (GC-IR), and pyrolysis GC-MS. This research will provide methods to identify cyclopropyl-containing synthetic cannabinoids, as well as answer what is happening to create two peaks in the chromatogram.

2. Materials and Method
2.1 Chemicals

Standards of UR-144, XLR-11, and XLR-11 4-pentenyl analog (4-PA), and 1ml of 1mg/ml XLR-11 4-fluoropentyl isomer (4-FPI) in MeOH were purchased from Cayman Chemical Company (Ann Arbor, MI). Standard solutions were prepared by dissolving ~1mg of UR-144, XLR-11, or 4-PA with ~1mL methanol (MeOH) and 330μl of 4-FPI with 300μl MeOH.

2.2 Heating Standards

Placed ~1mg of standard (330μl 4-FPI) in a well on a spot plate and covered with a watchglass. A hot plate was set to 300 ºC and allowed to heat for 10 minutes. The spot plate was placed on the hot plate for approximately 15 minutes, then removed and allowed to cool to room temperature. Both the spot plate and watchglass were rinsed with MeOH, collected in a test tube, and then evaporated until ~0.40ml remained.

2.3 Instrument Parameters

**GC-MS With 5% Phenyl Column:** An Agilent 7890A series gas chromatograph coupled with an Agilent 5975C XL series mass selective detector (MSD). For each sample, a single injection volume of 1.0μL was introduced into the instrument at a 100:1 split ratio. The column leading to the MSD was an Agilent Technologies (Santa Clara, CA) HP-5MS (30mX250.00μmX0.25μm). Helium carrier gas flow rate was a constant 2.0mL/min. The GC oven temperature began at 220ºC, held for 2 minutes, increased to 280ºC at 5ºC/min, and then held at 280ºC for 2 minutes. Mass spectra were obtained in scan mode in the range of m/z 34-550 with the electron ionization energy set at 70eV. The MS solvent delay was 2.00 minutes.

**GC-MS With 35% Phenyl Column:** An Agilent 6890 series GC coupled with an Agilent 5975C XL series MSD. For each sample, a single injection volume of 1.0μL was introduced into...
the instrument at a 100:1 split ratio. The column leading to the MSD was a Restek (Bellefonte, PA) Rxi-35Sil MS (30mX250.00µmX0.25µm). Helium carrier gas flow rate was a constant 2.0mL/min. The GC oven temperature began at 220ºC, held for 2 minutes, increased to 280ºC at 5ºC/min, and then held at 280ºC for 2 minutes. Mass spectra were obtained in scan mode in the range of m/z 34-550 with the electron ionization energy set at 70eV. The MS solvent delay was 1.65 minutes.

**LC-MS:** Agilent Technologies 1100 Series liquid chromatography (LC) with a mixture of 15mM ammonium acetate pH~4 and ACN (35:65 v/v) was used as mobile phase at a flow rate of 500.00µl/min. The LC was coupled with an Agilent Technologies 1100 series MSD in atmospheric pressure electro- spray (API-ES) ionization mode and LC/MSD ChemStation (2003) from Agilent Technologies was used. The detector voltage was set at 3000V. The acquisition range was from 100 to 650.

**Solid Phase GC-IR:** An Agilent 7890A GC series GC coupled with a Spectra Analysis (Greenville, SC) DiscovIR-GC. For each sample, a single injection volume of 1.0µL was introduced into the instrument at a 100:1 split ratio. The column leading to the MSD was a Restek (Bellefonte, PA) Rxi-35Sil MS (30mX250.00µmX0.25µm). Helium carrier gas flow rate was a constant 2.0mL/min. The GC oven temperature began at 220ºC, held for 2 minutes, increased to 280ºC at 5ºC/min, and then held at 280ºC for 2 minutes. Infrared spectra were obtained keeping the transfer line, oven, and restrictor were 250ºC. The Dewar cap was 30ºC and the disk was -40ºC. The chamber was kept at 1.00x10^{-4}torr.

**FT-IR:** Thermo Scientific (West Palm Beach, FL) Nicolet 6700 Fourier Transform – Infrared (FT-IR) equipped with a deuterated triglycine sulfate and thermoelectricity cooled
(DTGS TEC) detector and KBr beamsplitter. The following was used: 4.000 resolution, 2.0 sample gain, 0.6329 optical velocity, 74.00 aperture, and IR source.

Pyrolysis GC-MS: CDS Analysis (Oxford, PA) 5150 Pyroprobe® with a temperature program of 0°C (0sec) - 10.00°C/msec - 300°C (15sec). The interface program was 50°C (0min) - 0°C/min - 300°C (1.00min). The valve oven and transfer line were 300°C. The pyroprobe was connected to an Agilent 7890 GC series GC coupled with an Agilent 5975C XL series MSD. For each sample, a single injection volume of 1.0µL was introduced into the instrument at a 10:1 split ratio. The column leading to the MSD was an Agilent Technologies (Santa Clara, CA) HP-5MS (30mX250.00µmX0.25µm). Helium carrier gas flow rate was a constant 2.0mL/min. The GC oven temperature began at 220°C, held for 2 minutes, increased to 280°C at 5°C/min, and then held at 280°C for 2 minutes. Mass spectra were obtained in scan mode in the range of m/z 50-550 with the electron ionization energy set at 70eV.

3. Results and Discussion

After every spectrum was analyzed, any peak that pertained to the original molecule was labeled Standard and the other peak was labeled Product 1.

3.1 GC-MS With a 5% Phenyl Column
Figure 4. UR-144 Unheated using a 5% phenyl column. Chromatogram: (A) Standard Rt = 9.34min and Product 1 was very small (close-up in box) Rt = 9.77min. Mass Spectra: (B) Standard and (C) Product 1.
Figure 5. UR-144 Heated using a 5% phenyl column. Chromatogram: (A) Standard Rt = 9.31 min and Product 1 Rt = 9.77 min. Mass Spectra: (B) Standard and (C) Product 1.

The retention times for both the Standard and Product 1 peaks in the UR-144 Unheated (Fig. 4A) and the Heated (Fig. 5A) chromatogram differed by at most 0.02 min. The mass spectrum of the Unheated Standard (Fig. 4B) was compared to the mass spectrum of the Heated Standard (Fig. 5B). There were twenty-seven fragments in common, the parent and the base fragments were the same, and the ratios of those fragments were identical. When the mass spectra of the Product 1 (Fig. 4C and Fig. 5C) were compared there were twenty fragments in
common, the ratios of the fragments were close, and the same base and parent fragments. Based on the nearly identical retention times and the mass spectra, it was concluded that the Standard peak in both the Unheated and the Heated chromatograms were from the same molecule. The same conclusion was made for the Product 1. The larger ratio of the Product 1 peak to the Standard peak in the Heated chromatogram was the only difference between the Unheated and Heated samples.

The mass spectra of XLR-11, 4-FPI, and 4-PA are similar to Figure 4 and Figure 5. The retention time, peak area, and major ions of each drug is shown in Table 1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rt (min)</th>
<th>Peak Area</th>
<th>Total Percent Area (%)</th>
<th>Major Ions (m/z) [Relative Abundance (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-PA Unheated</td>
<td>9.32 [S]</td>
<td>87474342</td>
<td>99.4</td>
<td>212*, 226 [4], 294 [17], 309 [14]**</td>
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<tr>
<td></td>
<td>9.73 [P]</td>
<td>550094</td>
<td>0.6</td>
<td>172 [21], 212*, 226 [37], 294 [58], 309 [28]**</td>
</tr>
<tr>
<td>4-PA Heated</td>
<td>9.30 [S]</td>
<td>13979056</td>
<td>91.2</td>
<td>212*, 226 [3], 294 [15], 309 [14]**</td>
</tr>
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<td></td>
<td>9.73 [P]</td>
<td>1356562</td>
<td>8.8</td>
<td>172 [18], 212*, 226 [44], 294 [47], 309 [25]**</td>
</tr>
<tr>
<td>4-FPI Unheated</td>
<td>9.89 [S]</td>
<td>35014227</td>
<td>98.8</td>
<td>232*, 247 [8], 314 [17], 329 [15]**</td>
</tr>
<tr>
<td></td>
<td>10.34 [P]</td>
<td>133554</td>
<td>0.4</td>
<td>172 [24], 232*, 247 [69], 314 [40], 329 [12]**</td>
</tr>
<tr>
<td></td>
<td>12.18 [C]</td>
<td>284908</td>
<td>0.8</td>
<td>41 [20], 212 [40], 248*, 263 [10], 330 [20], 345 [20]**</td>
</tr>
<tr>
<td>4-FPI Heated</td>
<td>9.87 [S]</td>
<td>2715291</td>
<td>97.7</td>
<td>232*, 247 [6], 314 [11], 329 [9]**</td>
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<td></td>
<td>10.33 [P]</td>
<td>28079</td>
<td>1.0</td>
<td>41 [27], 232*, 247 [82], 314 [18]**</td>
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<tr>
<td></td>
<td>12.18 [C]</td>
<td>36566</td>
<td>1.3</td>
<td>41 [13], 144 [17], 212 [38], 248***</td>
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<tr>
<td>XLR-11 Unheated</td>
<td>10.73 [S]</td>
<td>74954389</td>
<td>99.0</td>
<td>232*, 247 [9], 314 [15], 329 [12]**</td>
</tr>
<tr>
<td></td>
<td>11.19 [P]</td>
<td>791154</td>
<td>1.0</td>
<td>172 [25], 218 [13], 232*, 247 [81], 314 [44], 329 [23]**</td>
</tr>
<tr>
<td></td>
<td>11.21 [P]</td>
<td>32183810</td>
<td>96.4</td>
<td>172[21], 218 [10], 232*, 247 [81], 314 [43], 329 [22]**</td>
</tr>
</tbody>
</table>

S = Standard Peak  
P = Product 1 Peak  
C = Contamination Peak  
* = Base Ion  
** = Parent Ion  
*** = Base and Parent Ion  
N/A = Not seen
The 4-PA Unheated and the Heated (Table 1) Standard and Product 1 retention times for differed by at most 0.02min. The mass spectra of the Unheated and the Heated for the Standard had an identical number of fragments, base and parent fragments, and ratios of those fragments. There was a close ratio of the fragments, same base and parent fragments, and twenty common fragments for the Product 1. Based on the close retention times and the close mass spectra, it was concluded that the only difference between the Unheated and the Heated samples was the larger ratio of the Product 1 peak to the Standard peak in the chromatogram.

The 4-FPI Unheated and the Heated (Table 1) Standard retention time differed by 0.02min. Product 1 could not be identified in the Heated chromatogram. The mass spectra for the Standard had the same m/z was for the parent and the base fragments, twenty-nine common fragments, and the ratios of those fragments, minus m/z 212, were nearly identical. It was concluded that the small starting sample (330μl) was not enough to change the ratio between the Standard and the Product 1 in the chromatogram. It was also concluded that the same molecule was the Standard in both the Unheated and the Heated chromatograms.

The XLR-11 Unheated and Heated (Table 1) retention times for the Standard and Product 1 peaks differed by at most 0.04min. The mass spectra of the Standard had twenty-seven common fragments, the same parent and base fragments, and the ratios were identical. The Product 1 had the same base and parent fragments, twenty-five common fragments, and the ratios were nearly identical. Based on the close retention times and the mass spectra, it was concluded that the only difference between the Unheated and the Heated samples was the larger ratio of the Product 1 peak to the Standard peak in the Heated chromatogram.

Based on the close retention times, 4-PA and UR-144 could be co-eluting and therefore could be interfering with the mass spectra of each other.
3.2 GC-MS With a 35% Phenyl Column

A second GC-MS was used to observe the differences between columns and to try to separate the peaks better to prevent possible co-elution. The chromatograms and the mass spectra for each drug are similar to Figure 4 and Figure 5, therefore all the important data is displayed in Table 2.

Table 2. Retention times, peak areas, and major ions of the Unheated and Heated samples using a 35% phenyl column.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rt (min)</th>
<th>Peak Area</th>
<th>Total Percent Area (%)</th>
<th>Major Ions (m/z) [Relative Abundance (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>UR-144</td>
<td>9.88 [S]</td>
<td>75510719</td>
<td>99.4</td>
<td>214*, 229 [9], 296 [18], 311 [20]**</td>
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<tr>
<td>Unheated</td>
<td>10.56 [P]</td>
<td>460635</td>
<td>0.6</td>
<td>172 [24], 200 [4], 214*, 229 [89], 296 [77], 311 [34]**</td>
</tr>
<tr>
<td>UR-144</td>
<td>9.87 [S]</td>
<td>15368293</td>
<td>35.1</td>
<td>214*, 229 [7], 296 [18], 311 [20]**</td>
</tr>
<tr>
<td>Heated</td>
<td>10.58 [P]</td>
<td>28472029</td>
<td>64.9</td>
<td>172 [15], 200 [8], 214*, 229 [89], 296 [67], 311 [40]**</td>
</tr>
<tr>
<td>4-PA</td>
<td>10.06 [S]</td>
<td>10436386</td>
<td>99.1</td>
<td>212*, 226 [2], 294 [18], 309 [19]**</td>
</tr>
<tr>
<td>Unheated</td>
<td>10.74 [P]</td>
<td>969132</td>
<td>0.9</td>
<td>172 [19], 212*, 226 [44], 294 [59], 309 [37]**</td>
</tr>
<tr>
<td>4-PA</td>
<td>10.04 [S]</td>
<td>28997297</td>
<td>88.8</td>
<td>212*, 226 [3], 294 [23], 309 [23]**</td>
</tr>
<tr>
<td>Heated</td>
<td>10.74 [P]</td>
<td>3659526</td>
<td>11.2</td>
<td>172 [16], 212*, 226 [43], 294 [66], 309 [43]**</td>
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<td>4-FPI</td>
<td>10.89 [S]</td>
<td>61758441</td>
<td>96.3</td>
<td>232*, 247 [6], 314[15], 329 [16]**</td>
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<tr>
<td>Unheated</td>
<td>11.60 [P]</td>
<td>563147</td>
<td>0.9</td>
<td>172 [16], 232*, 247 [80], 314[59], 329 [36]**</td>
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<td></td>
<td>13.64 [C]</td>
<td>1833485</td>
<td>2.9</td>
<td>41 [17], 212 [33], 248*, 263 [11], 330 [28], 345 [28]**</td>
</tr>
<tr>
<td>4-FPI</td>
<td>10.87 [S]</td>
<td>10539752</td>
<td>96.0</td>
<td>232*, 247 [7], 314[16], 329 [17]**</td>
</tr>
<tr>
<td>Heated</td>
<td>11.60 [P]</td>
<td>179765</td>
<td>1.6</td>
<td>172 [36], 232 [73], 247*, 314[27], 329 [35]**</td>
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<td>253818</td>
<td>2.3</td>
<td>41 [14], 212 [43], 248*, 330 [21], 345 [32]**</td>
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<td>XLR-11</td>
<td>11.97 [S]</td>
<td>98171754</td>
<td>98.4</td>
<td>232*, 247 [9], 314 [19], 329 [19]**</td>
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<td>Unheated</td>
<td>12.69 [P]</td>
<td>1624684</td>
<td>1.6</td>
<td>172[4], 218 [9], 232*, 247 [91], 314 [61], 329 [39]**</td>
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<tr>
<td>XLR-11</td>
<td>11.94 [S]</td>
<td>5515567</td>
<td>10.4</td>
<td>232*, 247 [1], 314 [19], 329 [19]**</td>
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<tr>
<td>Heated</td>
<td>12.71 [P]</td>
<td>47366110</td>
<td>89.6</td>
<td>172[18], 218 [7], 232*, 247 [84], 314 [70], 329 [34]**</td>
</tr>
</tbody>
</table>

S = Standard Peak  
P = Product 1 Peak  
C = Contamination Peak  
* = Base Ion  
** = Parent Ion
The UR-144 Unheated and the Heated (Table 2) Standard and Product 1 retention times differed by at most 0.02min. The mass spectra for the Standard had the same base and parent fragments, twenty-eight common fragments, and nearly identical ratios. The Product 1 mass spectra had eighteen common fragments, the same parent and base fragments, and close ratios. Based on the nearly identical retention times and mass spectra, it was concluded that the only difference between the Unheated and the Heated samples was the larger ratio of the Product 1 peak to the Standard peak in the chromatogram.

The Unheated and the Heated 4-PA (Table 2) retention times for the Standard and Product 1 peaks differed by at most 0.02min. The Standard mass spectra had 28 common fragments, identical ratios, and the same base and parent fragments. The mass spectra for the Product 1 had close ratios, the same parent and base fragments, and twenty-three common fragments. Based on the close retention times and nearly identical mass spectra, it was concluded that the only difference between the Unheated and the Heated samples was the larger ratio of the Product 1 peak to the Standard peak in the chromatogram.

The Unheated and the Heated (Table 2) Standard and Product 1 retention times for 4-FPI differed by at most 0.02min. The mass spectra for the Standard had twenty-nine common fragments, nearly identical ratios, and the same base and parent fragments. The Product 1 mass spectra had fifteen common fragments, the parent fragments were the same, different base peaks (Unheated m/z=232, Heated m/z=247), and the ratios were different. Based on the close retention times and nearly identical mass spectra of the Standard, it was concluded that the same molecule was the Standard in both the Unheated and the Heated samples. It was also concluded that the small starting sample (330μl) was not enough to change the ratio between the Standard
and the Product 1 and it could not be concluded if the same molecule was Product 1 in both the Unheated and the Heated chromatograms.

The XLR-11 Unheated and the Heated (Table 2) retention times for the Standard and Product 1 peaks differed by at most 0.03min. The Standard mass spectra had twenty-five common fragments, the same parent and base fragments, and identical ratios. The mass spectra of the Product 1 had the same parent and base fragments, nearly identical ratios, and twenty-six common fragments. Based on the nearly identical retention times and mass spectra, it was concluded that the only difference between the Unheated and the Heated samples was the larger ratio of the Product 1 peak to the Standard peak in the chromatogram.

Based on the differing retention times, 4-PA and UR-144 were not co-eluting and therefore were not interfering with the mass spectra of each other. It was concluded that the only difference between columns was in the retention times.

3.3 LC-MS

Liquid chromatography was used to determine if the Unheated samples were contaminated. Since it was hypothesized that cyclopropyl bonds break when heated, an instrument that did not require heating to separate samples was necessary.
Table 3. Liquid Chromatography data for each sample. There was only one peak in each of the chromatograms, except 4-FPI had contamination.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rt (min)</th>
<th>Molecular Weight (g/mol)</th>
<th>[M + H⁺]</th>
</tr>
</thead>
<tbody>
<tr>
<td>UR-144 Unheated</td>
<td>2.21 [S]</td>
<td>312</td>
<td></td>
</tr>
<tr>
<td>UR-144 Heated</td>
<td>1.77 [P]</td>
<td>312</td>
<td></td>
</tr>
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<td>4.0.3 Heated</td>
<td>2.23 [S]</td>
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<td>310</td>
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<td>1.31 [S]</td>
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S = Standard Peak
P = Product 1 Peak
C = Contamination Peak

The retention time for the UR-144 Unheated and Heated (Table 3) Standard peaks differed by 0.02 min. The Heated had one additional peak that eluded before the Unheated. The molecular weights for the Unheated Standard, the Heated Product 1, and the Heated Standard were all 311. Based on the close retention times and the same molecular weight, it was concluded that the Unheated Standard and the Heated Standard were the same molecule.

The 4-PA Unheated and the Heated (Table 3) Standard peak retention times were exactly the same. The Heated had one additional peak that eluded before the Unheated. The molecular weights for the Unheated Standard, the Heated Product 1, and the Heated Standard were all 309. Based on the nearly identical retention times and the same molecular weights, it was concluded that the Unheated Standard and the Heated Standard were the same molecule.

In 4-FPI Unheated and Heated (Table 3) retention times for the Standard and Contamination peaks differed by at most 0.01 min. The Heated had one additional peak that
eluded before the Unheated. The molecular weights for the Unheated Standard, the Heated Product 1, and the Heated Standard were all 329. Based on the nearly identical retention times and the same molecular weights, it was concluded that the Unheated Standard and the Heated Standard were the same molecule.

The retention time for the XLR-11 Unheated and Heated (Table 3) Standard peaks differed by 0.01 min. The Heated had one additional peak that eluded before the Unheated. The molecular weights for the Unheated Standard, the Heated Product 1, and the Heated Standard were all 329. Based on the nearly identical retention times and the same molecular weights, it was concluded that the Unheated Standard and the Heated Standard were the same molecule.

The appearance of a second peak in the Heated samples confirmed that heating the samples either externally or in the injection port created the second peaks in the GC-MS.

3.4 Solid Phase GC-IR

The solid phase GC-IR was used to obtain an IR spectrum of both the Standard and the Product 1 peaks. Table 4 displayed the retention times for each sample. For a particular sample (e.g. UR-144) the IR spectrum for the Unheated Standard peak matched the spectrum for the Heated Standard peak and the same was true for the Product 1 peaks. Since heat did not change the IR spectra, only the Unheated spectra (Fig. 6) is shown.

<table>
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<tr>
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<td>Product 1 Rt (min)</td>
<td>Standard Rt (min)</td>
<td>Product 1 Rt (min)</td>
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<td>-</td>
<td>-</td>
<td>15.48</td>
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</table>
Figure 6. GC-IR spectra for the Unheated Standard peaks (A, C, E, G) and the Unheated Product 1 peaks (B, D, F, H) for each Unheated sample. The wavenumbers indicated by arrows are bands that are only in the Product 1 spectrum.
The UR-144 retention times (Table 4) for the Standard differed by 0.12 min and the Product 1 differed by 0.07 min. The IR spectrum of Standard (Fig. 6A) contained six bands (1726, 1415, 1377, 975, 819, and 663 cm$^{-1}$) that were not in the Product 1 (Fig. 6B) spectrum and there was one band at 892 cm$^{-1}$ that only the Product 1 contained. The IR spectrum for the Heated Standard contained five bands (1415, 1377, 975, 819, and 663 cm$^{-1}$) that were not in the Product 1 and the Product 1 contained one band at 892 cm$^{-1}$ that was not in the Standard. Based on the IR spectra, it was concluded that the Unheated Standard and Heated Standard were the same molecule. The same conclusion was reached for Unheated Product 1 and Heated Product 1.

The 4-PA retention times (Table 4) for the Standard differed by 0.11 min and the Product 1 differed by 0.06 min. The Unheated Standard IR spectrum (Fig. 6C) contained five bands (1415, 1377, 977, 819, and 664 cm$^{-1}$) that were not in the Unheated Product 1 spectrum (Fig. 6D) and there was one band at 894 cm$^{-1}$ that only the Product 1 contained. The IR spectrum of the Heated Standard contained five bands (1415, 1377, 977, 819, and 665 cm$^{-1}$) that were only in the Standard and the Heated Product 1 contained one band at 893 cm$^{-1}$ that was not in the Standard. Based on the IR spectra, it was concluded that the Unheated Standard and the Heated Standard were the same molecule. The same conclusion was reached for the Unheated Product 1 and the Heated Product 1.

The 4-FPI retention times (Table 4) for the Standard differed by 0.09 min and the Product 1 differed by 0.06 min. The Unheated Standard IR spectrum (Fig. 6E) contained five bands (1415, 1378, 977, 817, and 663 cm$^{-1}$) that were not in the Unheated Product 1 spectrum (Fig. 6F) and there was one band at 895 cm$^{-1}$ that only the Product 1 contained. The IR spectrum of the Heated Standard contained five bands (1415, 1378, 977, 817, and 662 cm$^{-1}$) that were not in the Heated Product 1 and the Product 1 contained one band at 894 cm$^{-1}$ that was not in the Standard.
Based on the IR spectra, it was concluded that the Unheated Standard and the Heated Standard were the same molecule. The same conclusion was reached for the Unheated Product 1 and the Heated Product 1.

Both the XLR-11 Unheated and the Heated (Table 4) had only one peak in the chromatogram and the retention times differed by 0.845min. The IR spectrum of the Unheated (Fig. 6G) contained six bands (1415, 1377, 1108, 975, 819, and 664 cm⁻¹) that were only in the Unheated and the Heated (Fig. 6H) had one band at 893cm⁻¹ that was not in the Unheated IR spectrum. Based on the IR spectra, it was concluded that the Unheated was the Standard and the Heated was the Product 1.

The peaks between 885-895cm⁻¹ were interpreted as the C=CH₂ functional group. Since every Standard spectra did not contain a peak between 885-895cm⁻¹ and Product 1 did, it was concluded that only the Product 1 contained a C=CH₂ functional group.

3.5 FT-IR

The UR-144 FT-IR spectrum (Fig. 7A) was compared to the UR-144 Unheated Standard IR spectrum (Fig. 6A). The FT-IR spectrum contained two bands (1336 and 1177 cm⁻¹) that were not in the Standard spectrum. Based on the similarities between the IR spectra, it was concluded that the major component in UR-144 had the same structure as the Standard molecule.
The XLR-11 FT-IR spectrum (Fig. 7B) was compared to the XLR-11 Unheated Standard IR spectrum (Fig. 6C). The FT-IR spectrum contained three bands (1704, 1229 and 939 cm\(^{-1}\)) that were not in the Standard spectrum. Based on the similarities between the IR spectra, it was concluded that the main molecule in XLR-11 had the same structure as the Standard molecule.

3.6 Pyrolysis GC-MS

Pyrolysis GC-MS was used to observe what would happen to the Unheated if they were heated to 300ºC without the presence of oxygen.
Figure 8. UR-144 Pyrolysis GC-MS. Chromatogram: (A) Peak 1 Rt = 3.514, Peak 2 Rt = 3.961, Peak 3 Rt = 4.392, and Peak 4 Rt = 4.675. Mass Spectrum: (B) Peak 1, (C) Peak 2, (D) Peak 3, and (E) Peak 4.
The UR-144 chromatogram (Fig. 8A) contained four peaks. The mass spectra of each peak in the chromatogram were compared to the UR-144 Unheated mass spectra of the Standard and Product 1 (Fig. 4B and 4C). The mass spectra of both Peak 3 and Peak 4 had a parent peaks of m/z=345. Since the previous instruments did not give any indication that the UR-144 standard was contaminated, it could be concluded that the contamination was from the column. If the parent fragment for Peak 3 had been m/z=311, the mass spectrum of Peak 3 (Fig. 8D) would have been nearly identical to Figure 4B. If the parent fragment for Peak 4 had been m/z=311, the mass spectrum of Peak 4 (Fig. 8E) would have been nearly identical to Figure 4C. Had the parent fragment for both Peak 3 and Peak 4 been m/z = 311, it could have been concluded based on the rest of the spectrum, that Peak 3 was the same molecule as UR-144 Standard and that Peak 4 was the same molecule as UR-144 Product 1. Peak 1 and Peak 2 remained unknown.
Figure 9. XLR-11 Pyrolysis GC-MS. Chromatogram: (A) Peak 1 Rt = 3.994, Peak 2 Rt = 4.5, Peak 3 Rt = 5.032, and Peak 4 Rt = 5.366. Mass Spectrum: (B) Peak 1, (C) Peak 2, (D) Peak 3, and (E) Peak 4.
The XLR-11 chromatogram (Fig. 9A) contained three easily visible peaks and there was a small peak for Peak 2. The mass spectrum of each peak was compared to the XLR-11 Unheated mass spectra for the Standard and Product 1 (Table 1). The mass spectrum of Peak 3 (Fig. 9D) contained the same major ions and had close relative abundances to the XLR-11 Standard. The mass spectrum of Peak 4 (Fig. 9E) contained the same major ions and had close relative abundances to the XLR-11 Product 1. It was concluded that Peak 3 was the same molecule as XLR-11 Standard and that Peak 4 was the same molecule as XLR-11 Product 1. Peak 1 and Peak 2 remained unknown.

3.7 Structural Schemes for Fragment with M/Z 229

Scheme 1. A plausible scheme to explain the relative abundance of m/z 229 for the DM1P side chain of UR-144.
Scheme 2. A plausible scheme to explain the relative abundance of m/z 229 for the TMB product of UR-144.

Most of the fragments in the mass spectra of UR-144 Standard (Fig. 4A) and Product 1 (Fig. 4B) were the same but the ratio of those fragments differed. The relative abundance of the m/z 229 fragment changed the most by increasing from 7% in Standard to 64% in Product 1. Scheme 1 shows plausible electron pushing in which the DM1P side chain (Fig. 3C) of UR-144 broke two bonds to form the m/z 229 fragment. Scheme 2 showed plausible electron pushing in which the TMB product (Fig. 3B) of UR-144 broke one bond to form the m/z 229 fragment. It was concluded that Scheme 2 was more probable, since it would require less energy to break one bond and therefore more fragments could be formed, creating a higher relative abundance of m/z 229.

4. Conclusion

Manufactures have already circumvented the new law that controls general chemical structures, by designing new synthetic cannabinoids that contain cyclopropyl rings. UR-144 and XLR-11 are two of the synthetic cannabinoids that contain a cyclopropyl ring. These structures
are difficult to analyze on a GC-MS because the chromatogram contains multiple peaks and the mass spectra for those peaks have similar fragments.

It was hypothesized that the heat from the injection port caused the cyclopropyl ring to open, creating a new product. Both Unheated and Heated samples were analyzed using two GC-MSs, LC-MS, solid phase GC-IR, FT-IR, and pyrolysis GC-MS. The GC-MS revealed that the only difference between the Unheated and Heated samples was the ratio between the Standard peak and the Product 1 peak. The LC-MS showed that the molecular weight for each samples’ Standard peak was the same as the Product 1 peak. The solid phase GC-IR revealed that Product 1 contained a terminal double bond. The FT-IR confirmed that the major component in UR-144 and XLR-11 was the same structure as the respective Standard molecule. The pyrolysis GC-MS revealed more questions that need to be answered.

Based on the IR spectra, Product 1 contained a terminal double bond, therefore the DM2P (Fig. 3D) side chain could not be Product 1 since it contained C=C and not C=CH₂. Based on the relative abundance change of the m/z 299 fragment in UR-144, the DM1P (Fig. 3C) side chain could not be Product 1 since it would need to break two bonds to form the m/z 229 fragment. It was concluded that the Product 1 structure for all of the analyzed samples contained the TMB (Fig. 3B) side chain.

The best method to analyze the new synthetic cannabinoids would be to use a 35% phenyl column and an oven program that would start at 220°C, hold for 2 minutes, increase to 280°C at 5°C/min, and then hold at 280°C for 2 minutes. It was concluded that the second peak in the gas chromatogram resulted from the injection port thermally opening the cyclopropyl ring and possibly creating the products in Figure 10.
Figure 10. The probable Product 1 structures for each standard: (A) (1-pentyl-1H-indol-3-yl)(2,3,3-trimethyl-1-butene)methanone, (B) (1-(5-fluoropentyl)-1H-indol-3-yl)(2,3,3-trimethyl-1-butene)methanone, (C) (1-(pent-4-en-1-yl)-1H-indol-3-yl)(2,3,3-trimethyl-1-butene)methanone, and (D) (1-(4-fluoropentyl)-1H-indol-3-yl)(2,3,3-trimethyl-1-butene)methanone

4.1 Future Work

The first two peaks in the pyrolysis GC-MS chromatograms were unexpected and should be isolated and identified. The Open Top Product form of UR-144 (Fig. 33A) is currently being synthesized and will eventually be analyzed.

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References


