Characterization of a Cannabinomimetic Compound in Synthetic Cannabis by Gas Chromatography-High Resolution Time of Flight Mass Spectrometry (GC-HRT)

LECO Corporation; Saint Joseph, Michigan USA

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1. Introduction

Synthetic marijuana-type products have garnered a great deal of media attention over the last several months. Countless products often referred to as "incense" or "spice" are being sold at smoke shops, convenience stores, and online sites. These products are labeled "not for human consumption", but have been reported to have effects similar to cannabis when smoked. These reports have prompted testing in crime laboratories across the country. Some of the products tested have been confirmed to contain synthetic cannabinoids. The identification of these compounds by GC-MS can be challenging as most of them are not present in commercially available mass spectral search libraries.

This application note shows the analysis of a commercially available "incense" product that has since been removed from store shelves. The product, "Mr. Smiley", began to receive a great deal of media attention in the Midwestern United States since it was reported to induce a high similar to that of cannabis when smoked. The web community claimed that the product contained only natural ingredients, such as Damiana and Mullein Leaf. Authorities were quick to disagree, stating that they had reason to believe there were illegal compounds present.

Prior to authorities banning the sale of this product, it was purchased from a convenience store near Indianapolis, IN. The sample was taken to the LECO Life Science & Chemical Analysis Centre in St. Joseph, MI and was subsequently analyzed in an attempt to identify the presence of compounds that may be contributing to the "cannabis-like" highs. The sample was analyzed by gas chromatography time-of-flight mass spectrometry (GC-TOFMS). The most abundant analyte detected in the "incense" extract by GC-TOFMS was not present in the commercially available mass spectral search database (NIST 2008) which was used for spectral searching. Both electron ionization (EI) and chemical ionization (CI) experiments were conducted to verify the molecular ion. The LECO Pegasus® GC-HRT (High Resolution TOFMS) utilizing Folded Flight Path™ (FFP™) technology was used to acquire accurate mass data for the unknown compound. The data were used to perform a formula search which ultimately lead to the identification of a known cannabinoid receptor agonist.

2. Experimental Conditions

Samples

A 1 gram sample of the "Mr. Smiley" product was weighed into a 20 mL scintillation vial. A 10 mL aliquot of ethyl acetate was added and the sample was sonicated for 10 minutes. A portion of the ethyl acetate extract was transferred to GC autosampler vials for analysis. Samples were initially analyzed by GC-TOFMS on the LECO TruTOF® HT-TOFMS. Further characterization of an unknown analyte was performed using the LECO Pegasus GC-HRT high resolution mass spectrometer. The conditions for each system are included in the experimental section.

Experimental

GC-TOFMS (LECO TruTOF)

GC:	Agilent 7890
Injection:	1 µL, split 50:1 @ 275°C
Carrier Gas:	He @ 1.5 mL/min
Column:	
Rtx-5, 10 m x 0.18 mm x 0.2 µm (Restek Corporation, Bellefonte, PA)	
GC Oven:	60°C to 330°C @ 50°C/min
MS Transfer Line:	330°C
MS:	LECO TruTOF HT
Acquisition Delay:	60 seconds
Spectral Acquisition Rate:	20 spectra/s
Mass Range:	
40-550 m/z (EI), 50-550 m/z (CI)	
Electron Energy:	-70eV
Source Temperature:	250°C (EI), 200°C (CI)
CI Reagent Gas:	Methane

Instrument Control and Data Review: ChromaTOF®

Figure 1. LECO TruTOF HT (GC-TOFMS).

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The conditions used for the Pegasus GC-HRT analysis of the ethyl acetate extract of the "Mr. Smiley" product are shown below.

GC-HRTOFMS (LECO Pegasus GC-HRT)

GC:	Agilent 7890
Injection:	1μL, split 50:1@ 275°C
Carrier Gas:	He @ 1.5 mL/min
Column:	
	Rtx-1614, 20 m x 0.25 mm x 0.1 μm (Restek Corporation, Bellefonte, PA)
GC Oven:	60°C to 330°C @ 50°C/min
MS Transfer Line:	330°C

MS: LECO Pegasus GC-HRT (Electron Impact Ionization)

Acquisition Delay:	60 seconds
Resolution Mode:	High, 20m flight path
Spectral Acquisition Rate:	20 spectra/s
Mass Range:	40-550 m/z
Electron Energy:	-70eV
Source Temperature:	250°C

Instrument Control and Data Review:
ChromaTOF-HRT™

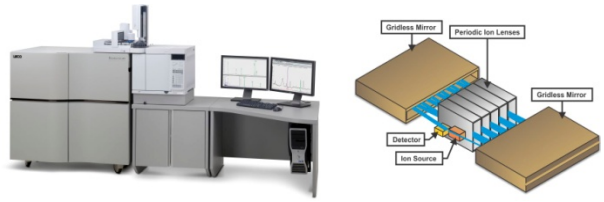


Figure 2. LECO Pegasus GC-HRT with Folded Flight Path (FFP) Technology.

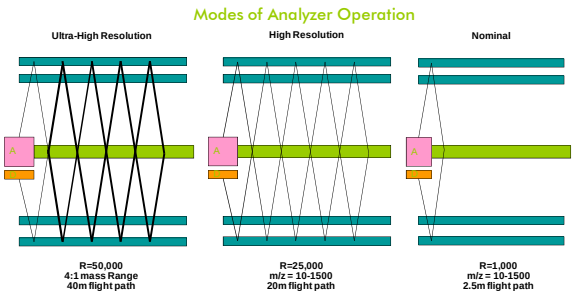


Figure 3. The image above provides a visual depiction of the various modes of operation made possible utilizing LECO's Folded Flight Path (FFP) technology. The Pegasus GC-HRT can operate in Ultra High (50,000 FWHM), High (25,000 FWHM), or Nominal (1,000 FWHM) mass resolution modes.

4. Results

GC-TOFMS Results

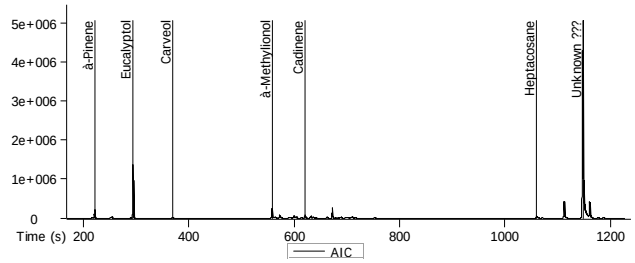


Figure 4. Analytical Ion Chromatogram (AIC) for ethyl acetate extract of "Mr. Smiley" product.

Figure 4 above shows the analytical ion chromatogram (AIC) from the GC-TOFMS analysis of an ethyl acetate extract of the "Mr. Smiley" product on the LECO TruTOF. Several analytes were identified in the ethyl acetate extract, but the most abundant analyte detected was not present in the commercially available library (NIST 2008) which was used to search the spectra of the detected compounds. The Peak True (deconvoluted) Electron Impact ionization (EI) mass spectrum for the unknown analyte is shown below in Figure 5. Though it appears that the molecular ion is shown at m/z 341, additional data was generated in CI mode using methane reagent gas for verification. The methane CI spectrum for the unknown analyte is shown in Figure 6. The spectrum contains a protonated molecular ion at m/z 342 as well as typical M+29 and M+41 adducts which support the molecular ion assignment of m/z 341. Further data were generated using the LECO Pegasus GC-HRT to provide an accurate mass based formula assignment. Those data are shown in the Pegasus GC-HRT results section of this application note.

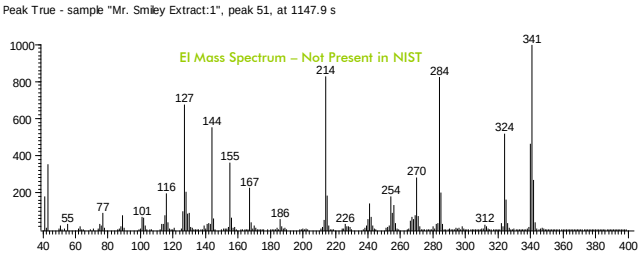


Figure 5. EI mass spectrum for unknown analyte in "Mr. Smiley" product.

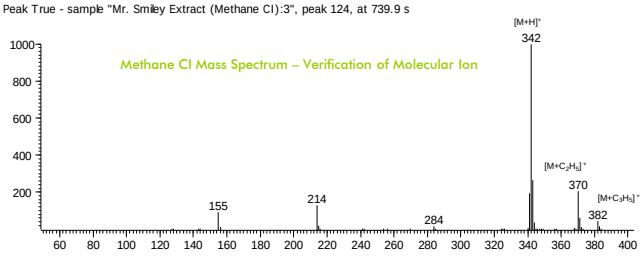


Figure 6. Methane CI mass spectrum for unknown analyte in "Mr. Smiley" product.

GC-HRT Results

The figure below (Figure 7) shows a total ion chromatogram (TIC) and extracted ion chromatogram for m/z 341.177 which is the observed accurate mass for the molecular ion of the unknown compound previously detected on the TruTOF unit mass resolution instrument. As stated earlier, this analyte was not present in the commercially available mass spectral search databases. As shown below in Figure 8, the observed accurate mass for the molecular ion of the unknown was 341.17675. A ChromaTOF-HRT formula search for the observed accurate mass suggested $C_{24}H_{23}NO$ as the #1 hit with a mass accuracy of -1.95 PPM relative to the theoretical exact mass for this formula. The resulting formula ($C_{24}H_{23}NO$) was consistent with a literature search which lead to the identification of this analyte as 1-pentyl-3-(1-naphthoyl)indole, or JWH-018, one of the most common synthetic cannabinoids used in "herbal incense" products. This compound was originally synthesized by Dr. John W. Huffman at Clemson University for use as a compound to study brain cannabinoid receptors.

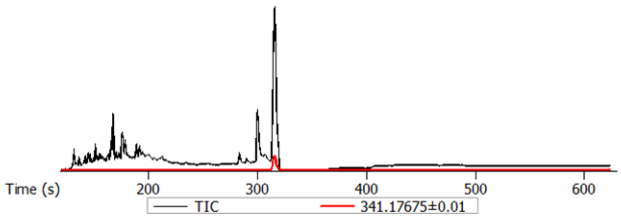
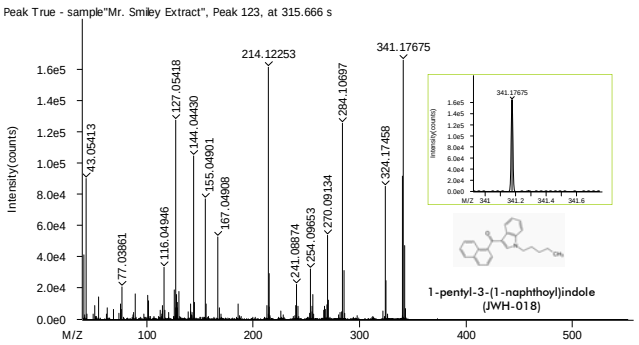


Figure 7. TIC "Mr. Smiley" and XIC for unidentified analyte.



Hit	Formula	Observed Ion m/z	Calculated Ion m/z	Mass Delta	Mass Accuracy (ppm)
1	C24H23NO	341.17675	341.177416	0.0007	-1.95

Figure 8. HRT Mass spectrum and accurate mass formula search results.

5. Conclusions

The Pegasus GC-HRT proved to be a valuable tool for the detection and identification of a synthetic cannabinoid in an herbal spice sample. The accurate mass data reported to <2 PPM provided the ability to successfully identify an unknown compound that was not present in the commercially available mass spectral search databases. The synthetic cannabis industry is growing rapidly, apparent by the sheer number of websites selling herbal spice samples. Legislation is being passed in many states to outlaw the distribution of these products. The problem is that there are literally hundreds of possible synthetic cannabinoid compounds, most of which are not present in the commercial mass spectral libraries. The use of a high resolution mass spectrometer capable of delivering the mass accuracy needed to facilitate unknown identification will be paramount to the ability of crime labs to effectively characterize these new and emerging compounds.

LECO Corporation • 3000 Lakeview Avenue • St. Joseph, MI 49085 • Phone: 800-292-6141 • Fax: 269-982-8977
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