Spice Wars–Are You Battle Ready? Analysis of Synthetic Cannabinoids via Gas Chromatography–High Resolution Time-of-Flight Mass Spectrometry

David E. Alonso[1], John Rorabeck[2], and Joe Binkley[1] | [1]LECO Corporation, St. Joseph, MI; [2]Berrien County Forensic Laboratory, Berrien Springs, MI

Introduction

Background

- Since the mid-2000s, synthetic drugs have been at the forefront of a world-wide market in "legal high" mind-altering substances sold to customers without proper manufacturing protocol, quality controls, general safety studies or dosing information.
- They are available over-the-counter or via the internet as plant fertilizer, incense, potpourri, or bath salts.
- They are not typically ordered on a routine lab test panel.
- They include the following series of compounds: JWH, CP, HU, AM, WIN, XLR, and UR (Fig. 1).

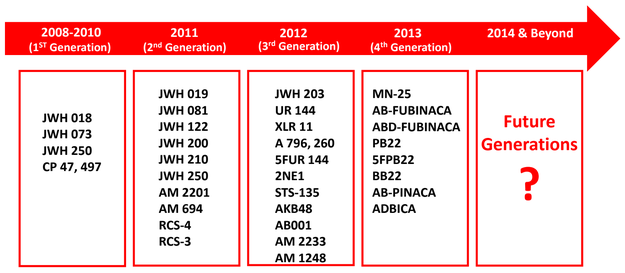


Fig. 1: Generations of Synthetic Cannabinoids

Analytical Challenges and The Solution

- Detection of designer drugs in clinical and forensic toxicology settings are complicated by:
  - Novelty of synthetic drugs (Moving targets)
  - Chemical diversity of compounds
  - Complexity of herbal samples (Botanical matrix, impurities, etc.)
  - Inappropriate sample preparation methodology
  - Unsuitable instrument analysis protocol
  - Lack of standards and/or absence of library spectra
- Solution:
  - Gas Chromatography–High Resolution (R>25,000), accurate mass (<1 ppm), time-of-flight mass spectrometry (GC-HRT)
  - High Resolution CI-Source (HR-CI) for complementary EI and CI data acquisition
  - Comprehensive data that can be interrogated multiple times as new drugs are discovered

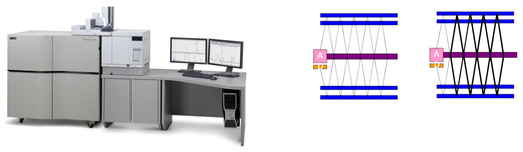


Fig. 2: LECO Pegasus® GC-HRT & Folded Flight Path® Mass Analyzer

Samples and Methods

- Case samples were obtained from a collaborating laboratory after they had been analyzed, and associated cases were completed.
- Sample Preparation:
  - Samples (30 mg) were placed in 20 mL scintillation vials. The botanicals were mixed with 3 mL of 2:1 $CHCl_3$/MeOH, vortexed for 1 min, sonicated for 3 min, and filtered into a 2 mL GC vial for analysis.

Workflow

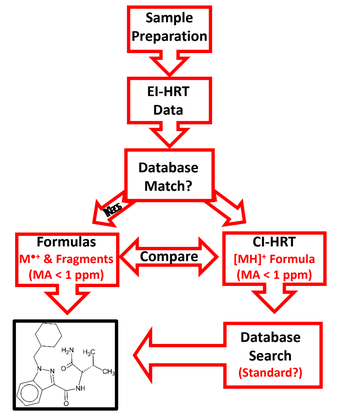


Fig. 3: Synthetic Drug Analysis Workflow

Table 1: GC-HRT Instrument Parameters

GC	Agilent 7890 with Gerstel MPS Auto Sampler
Column	Restek Rxi-5 MS (30m x 0.25mm x 0.25µm)
Carrier Gas, Flow	He, 1.5 mL/min Constant Flow
Injection	1 µL, Splitless
Inlet Temperature	270 ˚C
Temp. Program	50 °C (1 min) to 300 °C at 50 °C/min (5 min)
MS	**LECO Pegasus® GC-HRT**
Transfer Line Temp.	300 °C
Ion Source Temp.	EI 250 °C; CI 200 °C
Ionization	EI (70 eV); CI (140 eV)
Mass Range	EI 35 – 510; CI 60 - 510
Acquisition Rate	10 spectra/second
Calibration (Internal)	PFTBA
CI Reagent Gas	5% Ammonia in Methane

Results and Discussion

Mr. Nice Guy

- Synthetic cannabinoids, such as Mr. Nice Guy (JWH-018 & JWH-073), burst onto the world scene as first-generation synthetic cannabinoids. Figure 4 shows the analytical ion chromatogram (AIC) for Mr. Nice Guy, as well as the Peak True (Deconvoluted) mass spectra for JWH-073 & JWH-018.

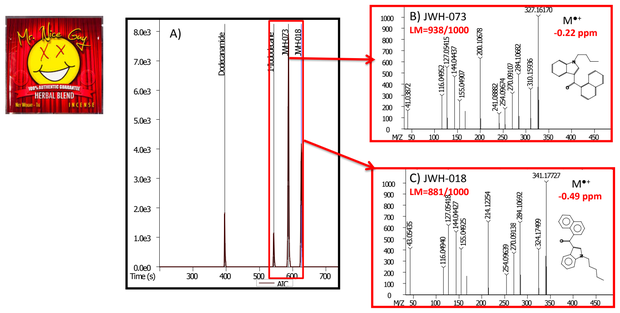


Fig. 4: A) Analytical Ion Chromatogram (AIC) of Mr. Nice Guy Extract. Peak True (Deconvoluted) Mass Spectra for B) JWH-073 and C) JWH-018

- In 2013, a seized drug packet was found to contain XLR-11, XLR-11 Isomer, and an unknown (Fig. 5). Acquisition of EI and CI-HRT data and a subsequent database search resulted in identification of the unknown as AB-Pinaca (Fig. 6).

Case 1: Botanical (Pokeweed)

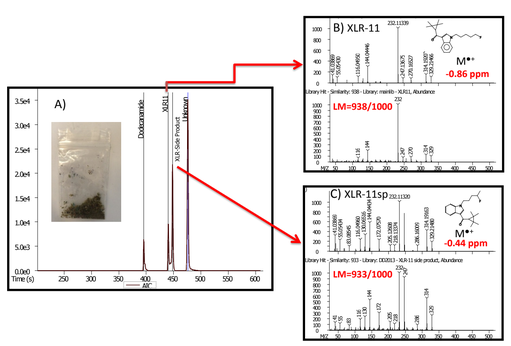


Fig. 5: A) Analytical Ion Chromatogram (AIC) of a Botanical Extract. Peak True Mass Spectra for B) XLR-11 and C) its 4-Fluoro Structural Isomer

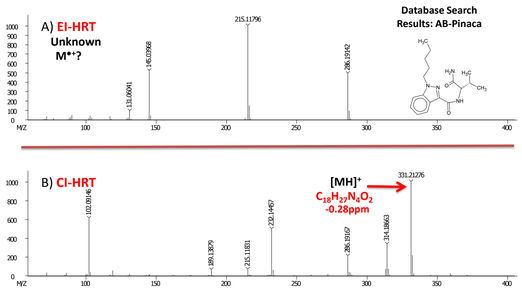
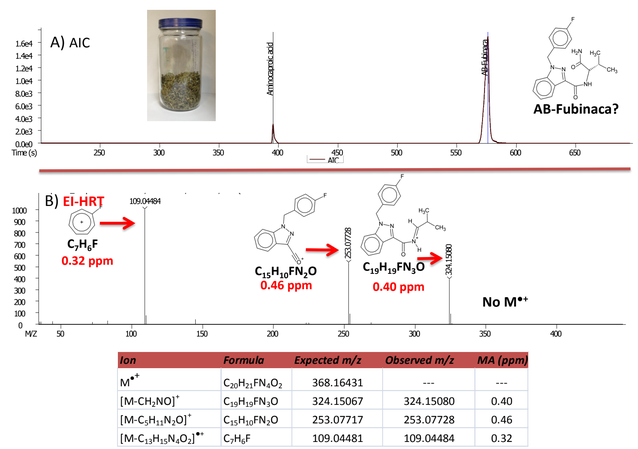


Fig. 6: A) Peak True EI-HRT and B) CI-HRT Mass Spectra for AB-Pinaca

Case 2: Confiscated Botanical Mixture

- A seized botanical material believed to be laced with synthetic cannabinoids was analyzed and resulted in the AIC and mass spectrum shown in Figure 7. This spectrum could not be matched to any in the Wiley 9, Wiley Designer Drug, or NIST 14 databases. A Cayman Chemical website search using the major fragment peaks m/z = 109, 253 resulted in AB-Fubinaca as a potential candidate for this unknown. The formulas for accurate mass fragments at m/z = 324.15080, 253.07728, and 109.04484 are listed in the table below (Ave. |ppm| = 0.39).



Ion	*Formula*	*Expected m/z*	*Observed m/z*	*MA (ppm)*
$M^{+\bullet}$	$C_{20}H_{21}FN_4O_2$	368.16431	---	---
$[M-CH_2NO]^+$	$C_{19}H_{19}FN_3O$	324.15067	324.15080	0.40
$[M-C_5H_{11}N_2O]^+$	$C_{15}H_{10}FN_2O$	253.07717	253.07728	0.46
$[M-C_{13}H_{15}N_4O_2]^{+\bullet}$	C_7H_6F	109.04481	109.04484	0.32

Fig. 7: A) Analytical Ion Chromatogram (AIC) of a Botanical Extract. B) Peak True Mass Spectra for a Compound Tentatively Assigned as AB-Fubinaca

- Acquisition of the corresponding CI-HRT data resulted in the protonated molecular ion at m/z = 369.17238 ($C_{20}H_{21}FN_4O_2$, 0.67 ppm), which was conclusive evidence for the synthetic cannabinoid AB-Fubinaca (Fig. 8).

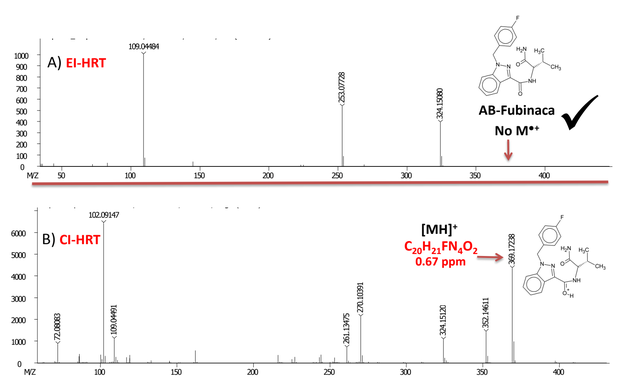
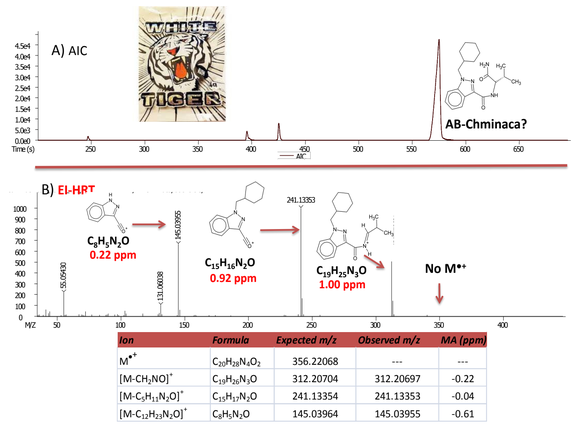


Fig. 8: A) Peak True EI-HRT and B) CI-HRT Mass Spectra for AB-Fubinaca

Case 3: Seized White Tiger Packet

- The AIC for an extract of a confiscated "White Tiger" packet is displayed below (Fig. 9). A database search of fragment ions (m/z -145, 241 and 312) in the mass spectrum of the major component suggested the sample consisted of AB-Chminaca—A new generation synthetic cannabinoid.



Ion	*Formula*	*Expected m/z*	*Observed m/z*	*MA (ppm)*
$M^{+\bullet}$	$C_{20}H_{28}N_4O_2$	356.22068	---	---
$[M-CH_3NO]^+$	$C_{19}H_{28}N_3O$	312.20704	312.20697	-0.22
$[M-C_5H_{11}N_2O]^+$	$C_{15}H_{17}N_2O$	241.13354	241.13353	-0.04
$[M-C_{12}H_{23}N_2O]^+$	$C_8H_5N_2O$	145.03964	145.03955	-0.61

Fig. 9: A) Analytical Ion Chromatogram (AIC) of a Botanical Extract and B) Peak True Mass Spectra for a Compound Tentatively Assigned as AB-Chminaca

- Supporting evidence for AB-Chminaca was obtained by comparing the EI and CI-HRT data (Fig. 10). The formula $C_{20}H_{29}N_4O_2$ (0.56 ppm) was calculated for the protonated molecular ion at m/z = 357.22870.

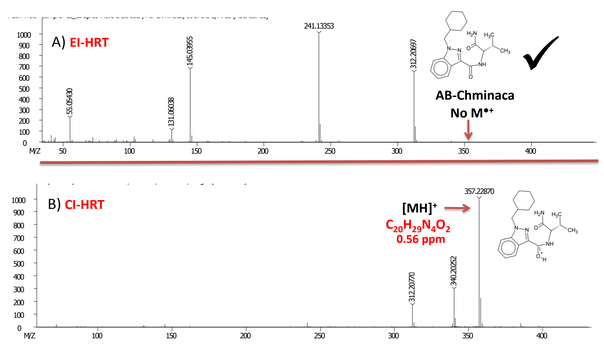


Fig. 10: A) Peak True EI-HRT and B) CI-HRT Mass Spectra for AB-Chminaca

Summary

- EI/CI workflow facilitates confident compound identification.
- GC-HRT and HR-CI source provides high quality, accurate mass data for:
  - Database searches (NIST, Wiley, etc.)
  - Formula determination (fragment, molecular, and adduct ions)