Spice Wars—Are You Battle Ready? Analysis of Synthetic Cannabinoids Using an HR-CI Source Operated in EI and CI Modes

LECO Corporation; Saint Joseph, Michigan USA

Key Words: Synthetic Cannabinoids, Electron and Chemical Ionization, High Resolution, Time-of-Flight Mass Spectrometry

1. Introduction

Since the mid-2000s, synthetic drugs have been at the forefront of a world-wide market in "legal high" consciousness-altering chemicals sold directly to consumers without any manufacturing standards, quality control, general safety assessments, or dosing information.[1] They are popular due to their novelty, initial "legal" status, and accessibility. Many are available over the counter, via the Internet, and are sold using deceptive descriptors such as plant fertilizer, incense, potpourri or bath salts.[2] These drugs are moving targets since they are easily modified and are typically not ordered on routine laboratory drug test panels. In this study, a workflow that includes rapid extraction, gas-chromatography high resolution time-of-flight mass spectrometry (GC-HRT) analysis, and identification of synthetic cannabinoids is described. For example, an Analytical Ion Chromatogram (AIC) for a seized drug packet (sample 1) was found to contain XLR-11, its 4-fluoro isomer, and an unknown (Figure 1). The XLR isomers were identified via a combination of database searches and molecular formula determinations using accurate mass data. Identification of the unknown, which at the time of analysis was not present in searched databases (NIST, Wiley), is detailed in the following results and discussion section (Section 3).

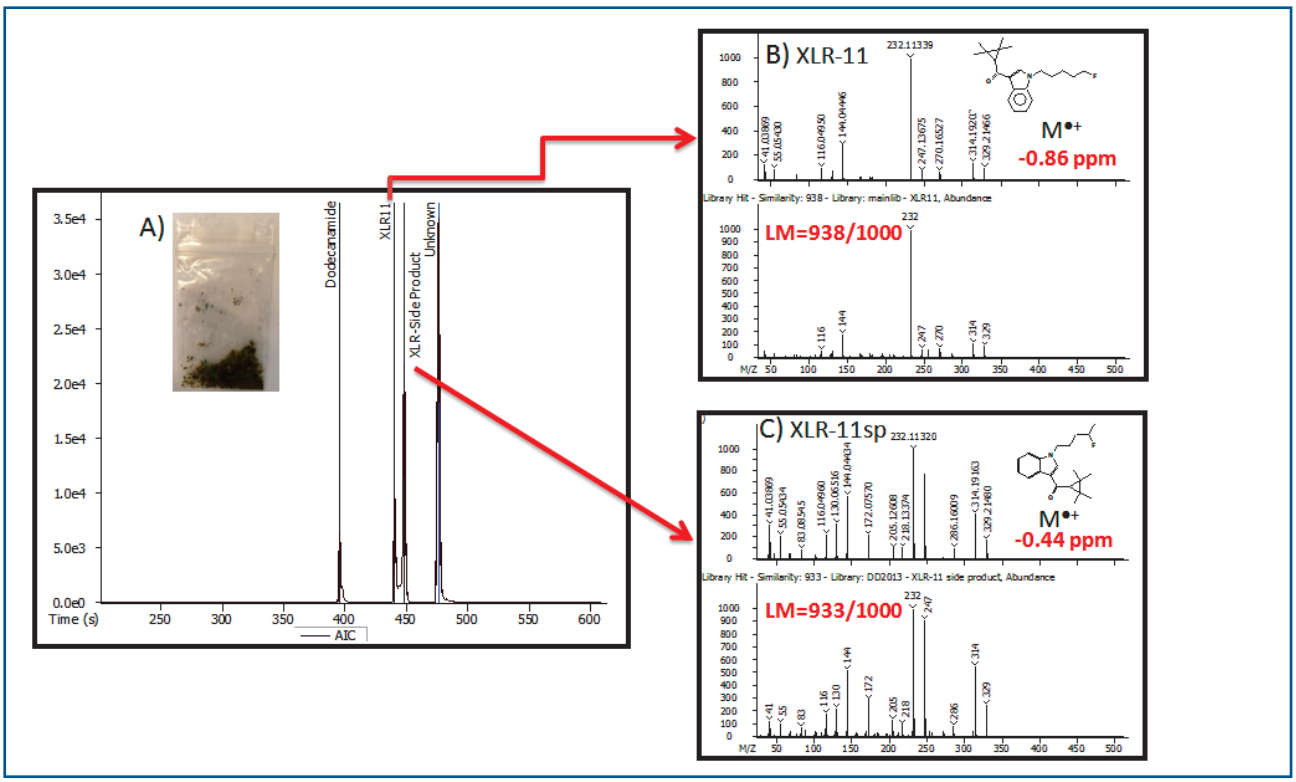


Figure 1. A) AIC of a botanical Extract Containing Several Synthetic Cannabinoids. Peak True Mass Spectra for B) XLR-11 and C) its 4-Fluoro Structural Isomer.

Life Science and Chemical Analysis Solutions

2. Experimental

Two samples were obtained from a collaborating forensic laboratory after they had been analyzed and associated cases were closed. The samples (30 mg) were placed in 20 mL scintillation vials and mixed with 3 mL of 2:1 $CHCl_3$/MeOH. The heterogenous mixtures were vortexed for 1 minute, sonicated for 3 minutes, and then filtered into 2 mL GC vials for analysis.

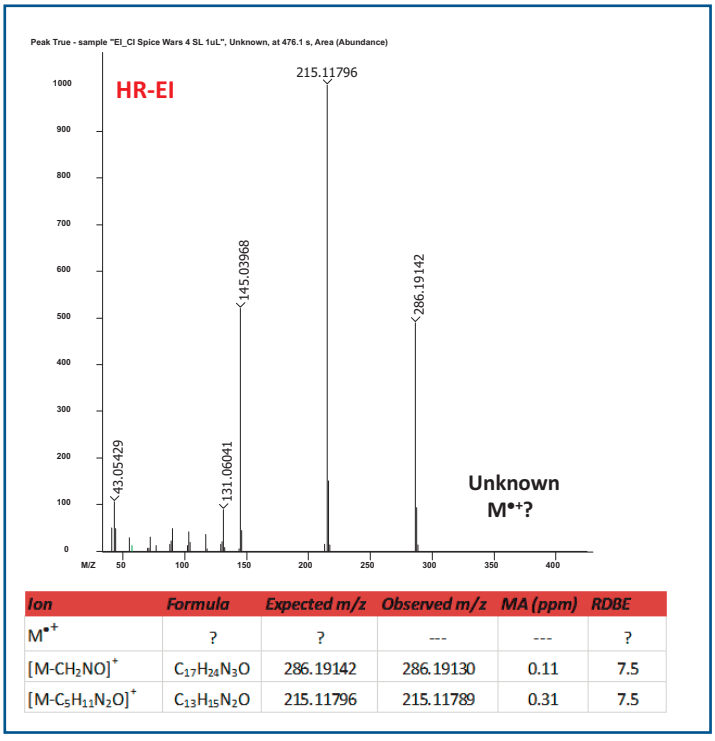
LECO's Pegasus® GC-HRT accurate mass (<1 ppm), high resolution (up to 50,000) instrument was used to analyze drug samples. Both electron and chemical ionization acquisitions were conducted using a High Resolution Chemical Ionization (HR-CI) source. Data was acquired and processed using ChromaTOF-HRT® brand software with High Resolution Deconvolution™ (HRD™). Spectral similarity searches were performed against Wiley and NIST libraries. Chemical formulas were automatically determined using accurate mass and isotopic pattern and spacing calculations.

Table 1. GC-High Resolution TOFMS (Pegasus GC-HRT) Conditions

Gas Chromatograph	**Agilent 7890 with MPS2 Autosampler**
Injection	1μL, Splitless @ 250°C
Carrier Gas	He @ 1.5 mL/min, Constant Flow
Column	Rxi-5 MS, 30 m x 0.25 mm i.d. x 0.25 μm (Restek, Bellefonte, PA, USA)
Temperature Program	50°C (1 min), to 300°C @ 50°C/min (5 min)
Mass Spectrometer	**LECO (Pegasus GC-HRT)**
Transfer Line	300°C
Ion Source Temperature	250°C (EI); 200°C (CI)
Acquisition Mode	High Resolution, R = 25,000 (FWHM)
Ionization Mode	EI (performed on CI Source) and CI (Reagent Gas: 5% NH_3 in CH_4)
Mass Range (m/z)	35-510 (EI Mode); 60-510 (CI Mode)
Acquisition Rate	10 spectra/s

3. Results and Discussion

EI-HRT analysis of the botanical sample 1 unknown resulted in the Peak True (Deconvoluted) mass spectrum displayed in Figure 2. Formula determinations for accurate mass ions at m/z = 286.19142 and 215.11789 resulted in $C_{17}H_{24}N_3O$ (Mass Accuracy (MA) = 0.11 ppm, RBDE = 7.5) and $C_{13}H_{15}N_2O$ (MA = 0.31 ppm, RBDE = 7.5) respectively. The non-integer RDBE values associated with these formulas were consistent with fragment ions. Therefore, CI-HRT data was acquired in order to determine the molecular formula for this unknown (Figure 3). A strong protonated molecular ion was observed at m/z = 331.21276 which corresponded to the formula $C_{18}H_{27}N_4O_2$ (MA = -0.28 ppm) and molecular formula of $C_{18}H_{26}N_4O_2$ (RBDE = 8.0).



Ion	*Formula*	*Expected m/z*	*Observed m/z*	*MA (ppm)*	*RDBE*
$M^{\bullet+}$	?	?	---	---	?
$[M\text{-}CH_2NO]^+$	$C_{17}H_{24}N_3O$	286.19142	286.19130	0.11	7.5
$[M\text{-}C_5H_{11}N_2O]^+$	$C_{13}H_{15}N_2O$	215.11796	215.11789	0.31	7.5

Figure 2. EI-HRT, Peak True Mass Spectra for Unknown in Botanical Sample (Top). Formulas, Mass Accuracy Values and RDBE Values for Ions at m/z = 286.19130 and 215.11789 (Bottom).

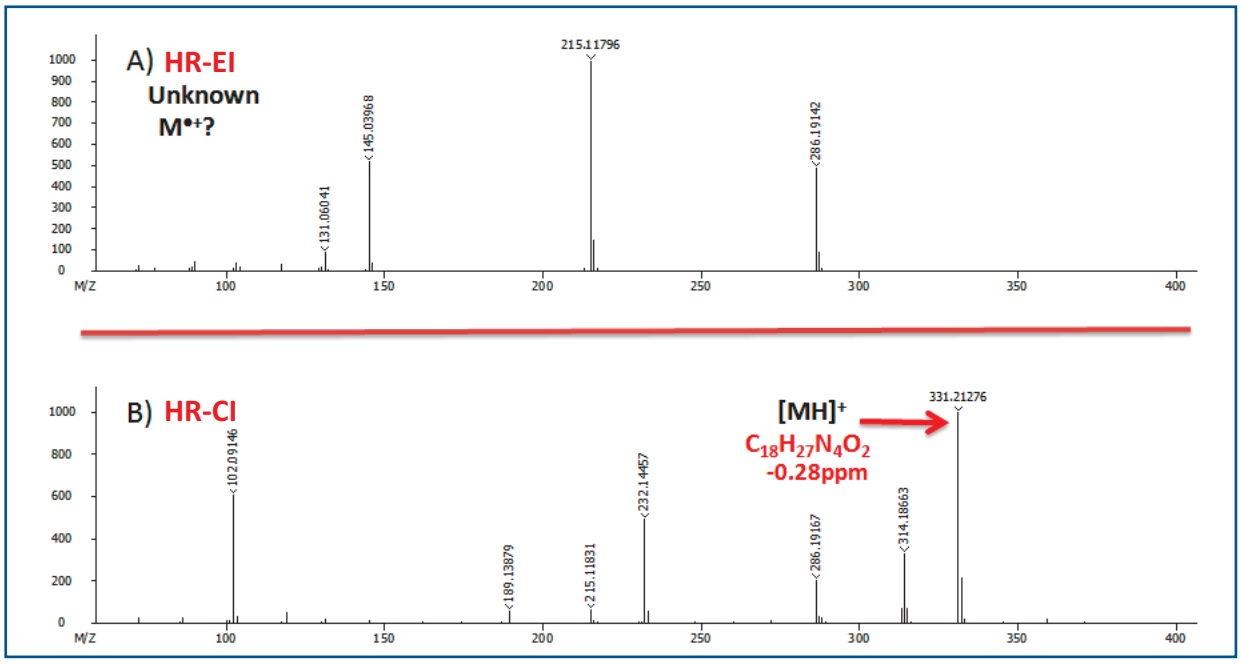


Figure 3. EI-HRT (A) and CI-HRT (B) Peak True Mass Spectra for Unknown in Botanical Sample.

Additional databases were explored since at the time of analysis, the unknown was not found in the original libraries used. For example, a Chemspider database[3] search of the formula $C_{18}H_{26}N_4O_2$ launched directly from ChromaTOF-HRT resulted in 2,332 hits with AB-Pinaca (id = 28537615) as one of the entries (Figure 4). This database is an excellent starting point for compound identification since it has a powerful search engine and contains over 32 million structures from hundreds of sources. The database is a valuable tool for the characterization of synthetic drugs given that many were initially designed for medical research. However, a more strategic approach is to narrow the search using smaller "drug-focused" databases such as those found at the Cayman Chemical[4] and Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)[5] websites. Indeed, AB-Pinaca was the number one hit out of three at Cayman Chemical and the lone hit at the SWGDRUG site.

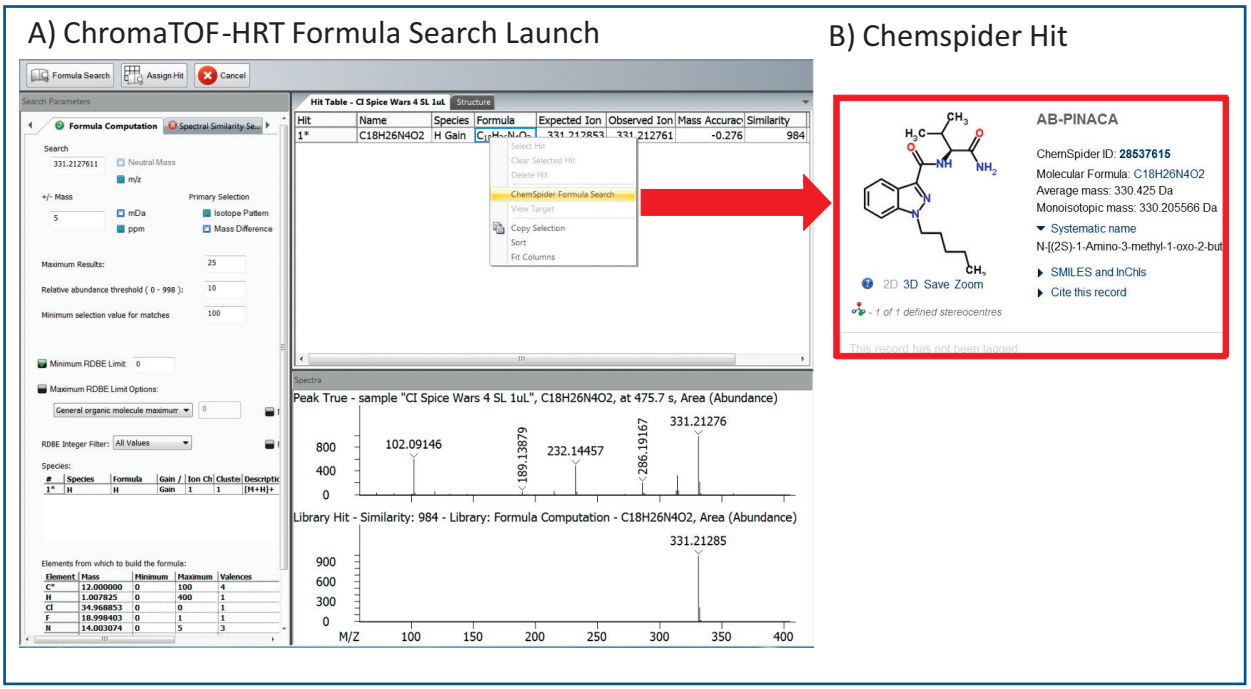
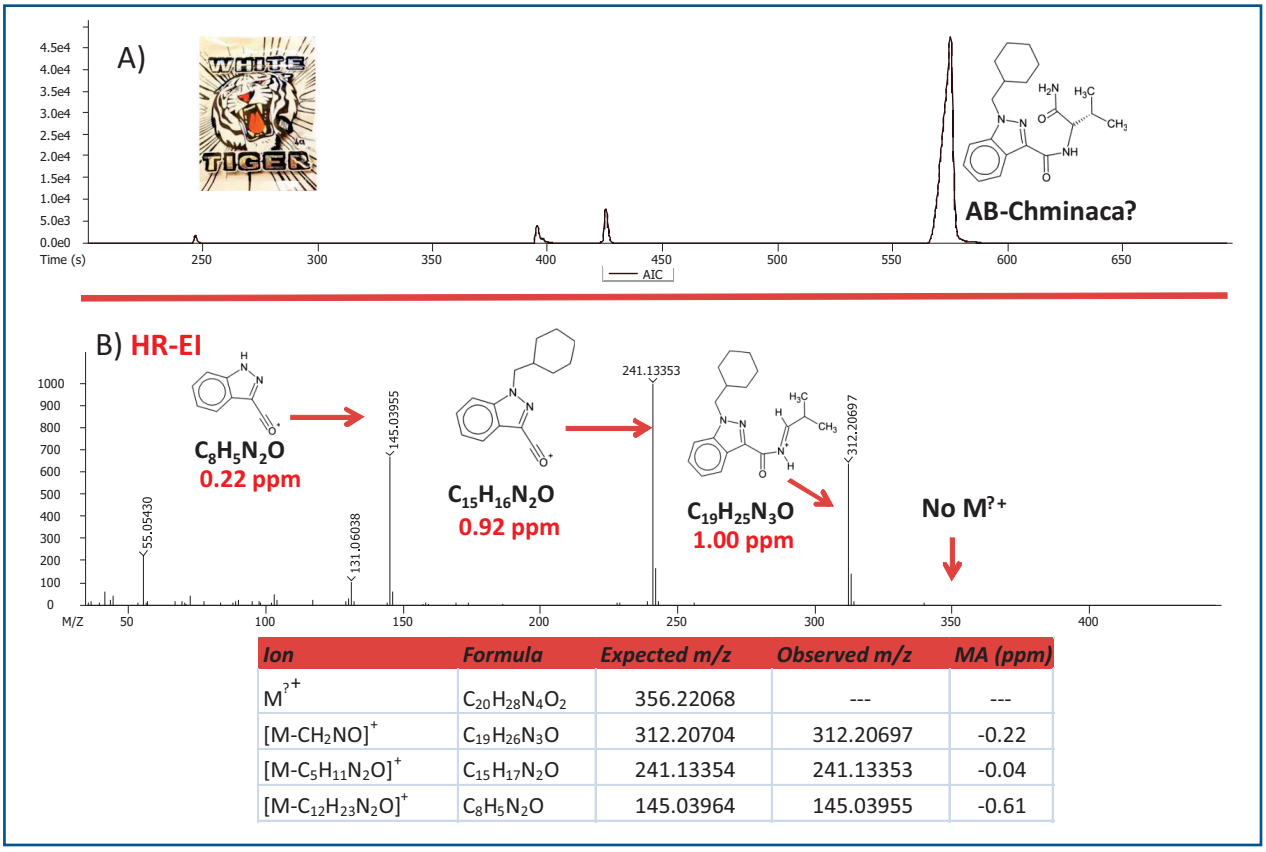


Figure 4. A) ChromaTOF-HRT Database Search Launch for $C_{18}H_{26}N_4O_2$ and B) one of the 2332 Hits From the Chemspider Database.

The AIC for an extract of a confiscated "White Tiger" packet (Sample 2) and the EI-HRT mass spectrum of its major component are displayed in Figure 5. This spectrum could not be matched to Wiley, Wiley Designer Drug, or NIST libraries. A Cayman Chemical website search of the major fragment peaks at 312, 241 and 145 suggested AB-Chminaca as a potential hit for the component. Formula determinations using accurate mass fragment ions at m/z = 312.20697 ($C_{19}H_{26}N_3O$, MA = -0.22 ppm), 241.13353 ($C_{15}H_{17}N_2O$, MA = -0.04 ppm), and (145.03955, MA = -0.61 ppm) provided support for the synthetic cannabinoid. The assignment of AB-Chminaca was confirmed through examination of the protonated molecular ion at m/z = 357.22870 ($C_{20}H_{29}N_4O_2$, MA = 0.56) in the complementary CI-HRT spectrum.



Ion	Formula	Expected m/z	Observed m/z	MA (ppm)
$M^{?+}$	$C_{20}H_{28}N_4O_2$	356.22068	---	---
$[M-CH_2NO]^+$	$C_{19}H_{26}N_3O$	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

Figure 5. A) EI-HRT AIC, B) Peak True Mass Spectra for Major Component in White Tiger. Formulas, Mass Accuracy Values and Structures for Fragment Ions at m/z = 312.20697, 241.13353 and 145.03955.

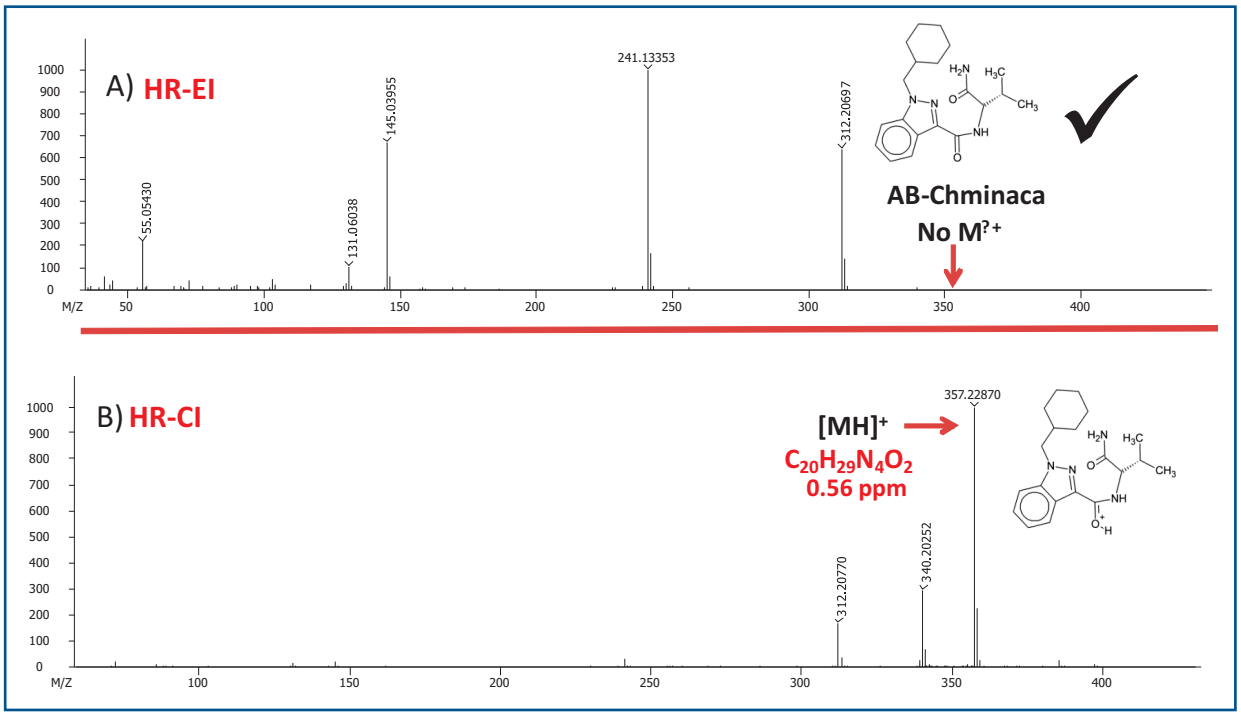


Figure 6. EI-HRT (A) and CI-HRT (B) Peak True Mass Spectra for Major Component in White Tiger.

4. Conclusion

High resolution, comprehensive (full mass range acquisition), accurate mass data is required to successfully fight the battle against synthetic drugs. This approach facilitates data mining and retrospective analysis of data for the presence of new compounds. LECO's *Pegasus* GC-HRT and HR-CI source provides high quality, accurate mass data for database searches and robust formula determinations for fragment, molecular, and adduct ions. The HR-CI source was utilized to successfully acquire both EI and CI data in subsequent injections allowing for a very efficient workflow for unknown compound identification. This EI/CI-HRT workflow facilitates quick and confident characterization of novel synthetic substances.

5. References

[1]Presley B.C., Jansen-Varnum S.A. and Logan B.K. "Analysis of Synthetic Cannabinoids in Botanical Material: A Review of Analytical Methods and Findings" *Forensic Science Review 2013*, 25, 27-46.

[2]Krasowski M.D, and Ekins S. "Using cheminformatics to predict cross reactivity of 'designer drugs' to their currently available immunoassays" *Journal of Cheminformatics 2014*, 6:22, 2014.

[3]http://www.chemspider.com/, accessed 12/2014.

[4]https://www.caymanchem.com/app/template/Home.vm, accessed 12/2014.

[5]http://www.swgdrug.org/, accessed 12/2014.

LECO Corporation | 3000 Lakeview Avenue | St. Joseph, MI 49085 | Phone: 800-292-6141 | Fax: 269-982-8977
info@leco.com • www.leco.com | ISO-9001:2008 | HQ-Q-994 | *LECO is a registered trademark of LECO Corporation.*

Life Science and Chemical Analysis Solutions