SHIMADZU
Excellence in Science

PO-CON1880E

High Resolution Designer Drug Screening using a High-Sensitivity Q-TOF Mass Spectrometer and an Extended Tandem Mass Spectrum Library

ASMS 2019

Jeff Dahl[1], Rachel Lieberman[1], Yuka Fujito[1],
Joseph Kahl[2] and Alex Giachetti[2]
[1]Shimadzu Scientific Instruments, Columbia Maryland,
[2]Miami-Dade Medical Examiner Department, Miami, FL

Introduction

The number of designer drugs and their availability has exploded in recent years, leading to widespread social impact. LC-MS is a powerful analytical technique to confirm which substances were involved in a particular toxicology case, however methods that detect only a few typical designer drugs cannot identify new or emerging drugs of concern. We developed a new LC-MS/MS method using a high-sensitivity Q-TOF mass spectrometer combined with an enhanced spectrum library to detect and screen for new designer drugs to support forensic investigations.

Figure 1 Typical dosage forms and packaging of designer drugs.

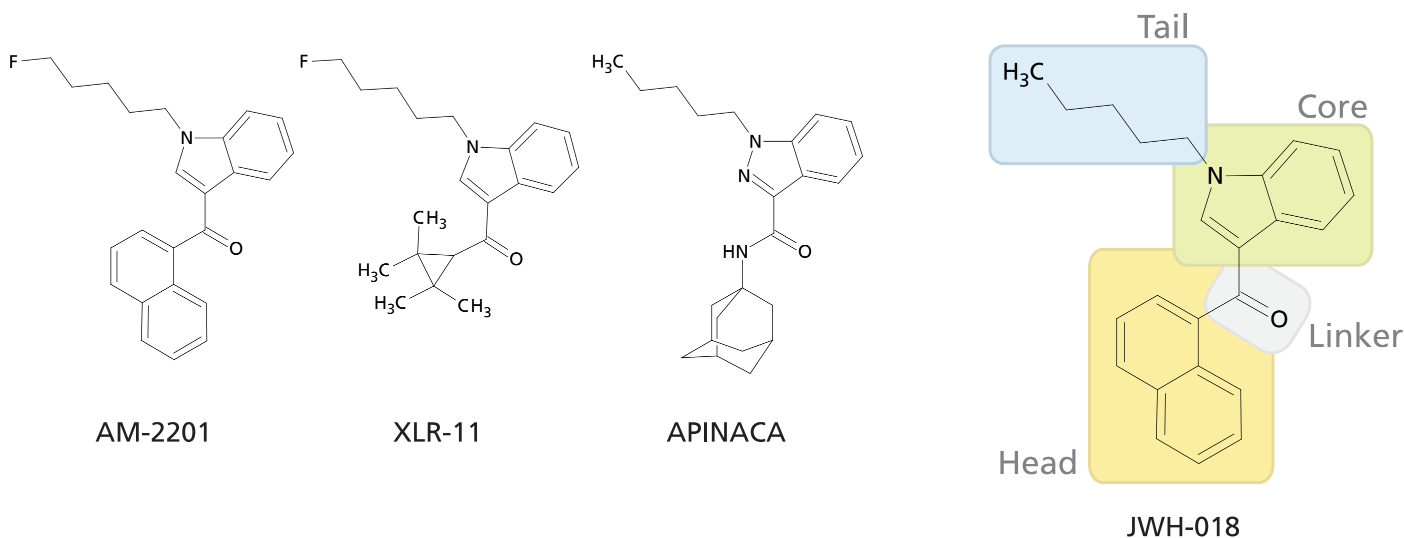


Figure 2 Structures of typical designer cannabinoids. Drug designers produce many variations on the same pharmacophore to evade regulations and testing.

Method

Authentic standards for emerging designer drugs were obtained and analyzed by a Q-TOF mass spectrometer to create a high resolution tandem mass spectrum library. Product ion spectra were obtained at eleven different fixed collision energies ranging from 10 to 60 eV, and additionally at a single collision energy of 35 eV with collision energy spreads of ±17 and ±25 eV.

Samples containing unknown designer drugs were prepared by solid phase extraction and analyzed by LC-MS/MS after centrifugation to remove particulates. Analysis was carried out using UHPLC separation, electrospray ionization, and detection in various MS modes including high resolution scan mode and data-dependent MS-MS.

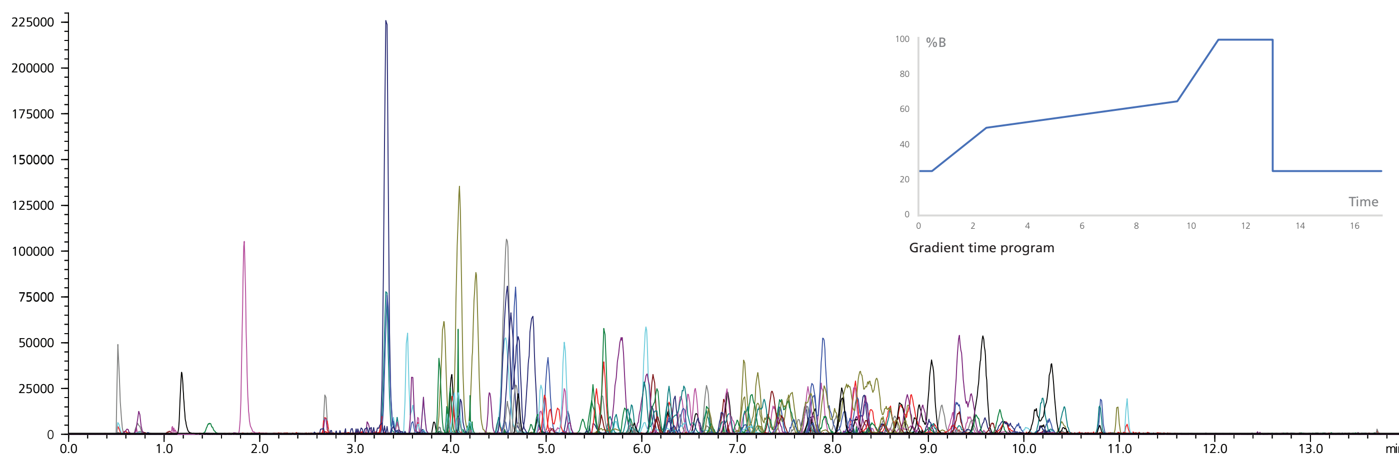


Figure 3 Extracted ion chromatograms of precursor ions corresponding to each compound in the library, from from MS1 scan data. From 350 compounds in the library, 185 unique precursor ions are observed.

Table 1 LCMS Analysis conditions

LC Column	: Raptor Biphenyl (2.1×100 mm, 2.7 µm)
Mobile Phase A	: 2 mM Amm. Fm. + 0.002% Formic Acid
Mobile Phase B	: Acetonitrile
Flow Rate	: 0.4 mL/min
Spray Voltage	: +4 kV
Interface Temp	: 300 °C
Nebulizing Gas	: 2 L/min
Drying Gas	: 10 L/min
DL Temp	: 250 °C
Heat Block Temp	: 400 °C

Results and Discussion

Accurate mass measurements of all precursor and product ions of authentic standards for library creation were within 1.5 ppm of the expected m/z, and in most cases the error was less than 0.8 ppm. Instrument resolving power was maintained above 30,000, as measured at m/z 922, over the duration of all measurements. For targeted analysis, the expected retention time was measured using authentic standards and used as part of the identification criteria. For untargeted analysis, scan mode and data-dependent MS-MS was used for analysis. Suspect peak lists were prepared by creating extracted ion chromatograms for m/z values of known formulas as well as by processing the MS data using untargeted feature extraction. Tandem mass spectra were compared to the newly created library spectra for tentative identification.

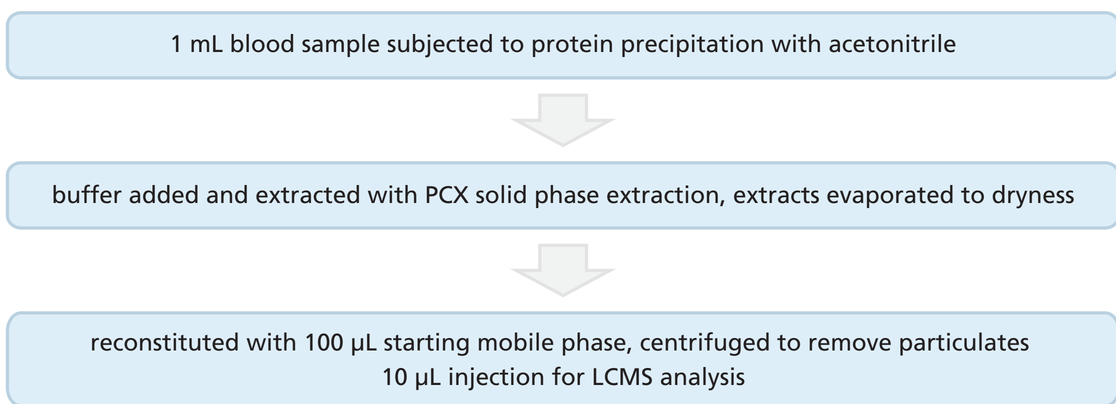


Figure 4 Sample preparation protocol

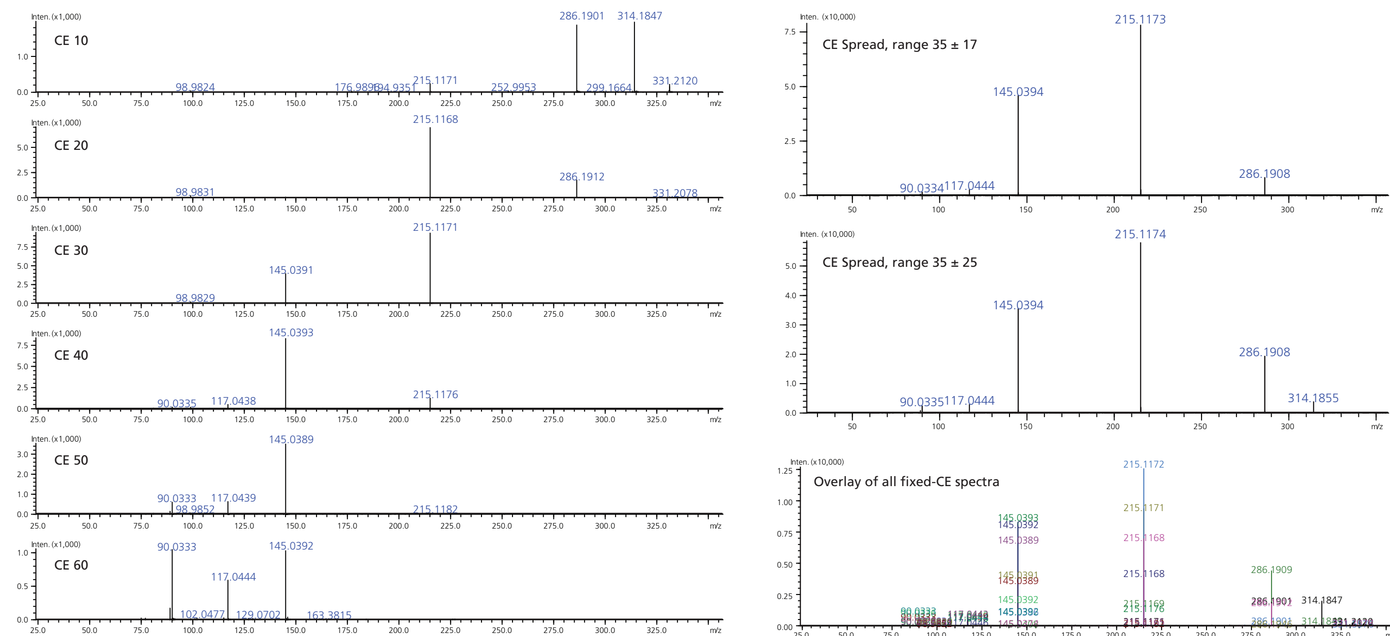
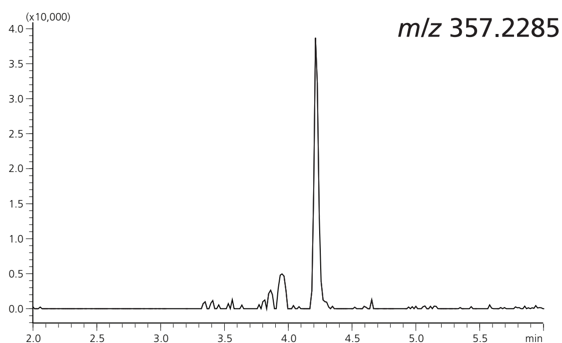


Figure 5 Using individual fixed-CE spectra, not all product ions can be represented. Using the CE Spread function, the complete range of product ions are observed. Fixed-CE and CE Spread data for authentic 3-Fluoro AMB are shown. Eleven fixed-CE values, from 10 eV to 60 eV in steps of 5, were used (selected values shown). Two CE Spread ranges were used. The CE Spread results compare closely with the overlaid fixed-CE spectra.

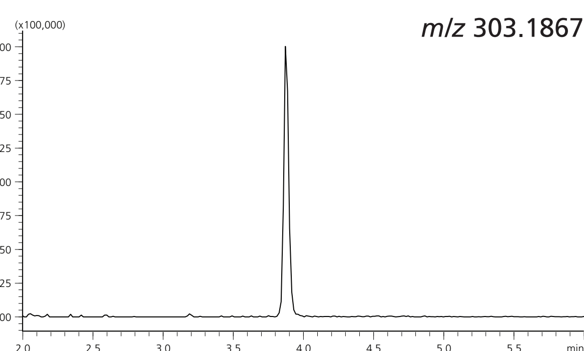
Table 2 Selected analytes detected in a set of ten post-mortem blood samples

Sample	Name
1	AB-CHMINACA
2	5F-PY-PICA
3	5-fluoro ADB metabolite 7
7	5F-ADB 5-fluoro ADB metabolite 7
8	5F-ADB 5-fluoro ADB metabolite 7
9	5-fluoro ADB metabolite 7
10	5F-ADB 5-fluoro ADB metabolite 7

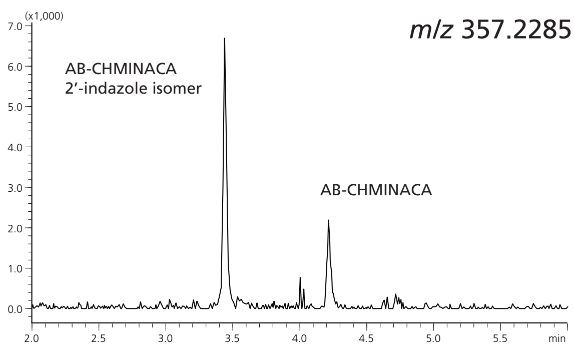
Post-mortem Sample 1



Post-mortem Sample 2



Authentic standard



Authentic standard

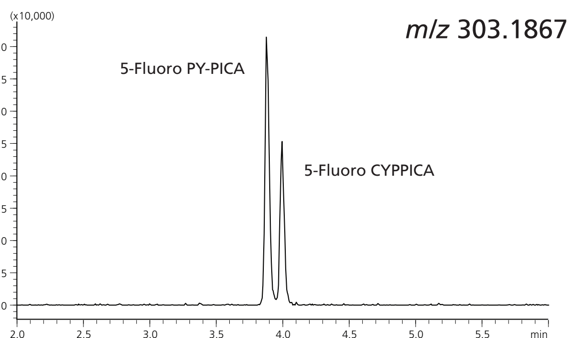
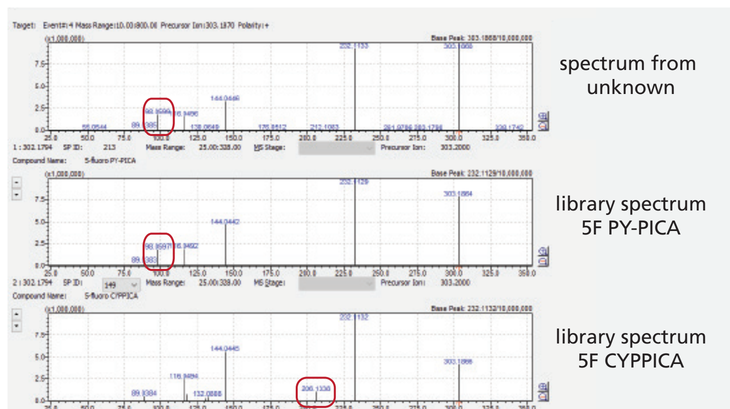


Figure 6 (Left) Mass chromatograms corresponding to AB-CHMINACA. The peak in post-mortem sample 1 matches AB-CHMINACA in *m/z*, retention time, and tandem mass spectrum. (Right) Mass chromatograms corresponding to 5-Fluoro PY-PICA. The *m/z*, retention time, and tandem mass spectrum matches the authentic standard.

High Resolution Designer Drug Screening using a High-Sensitivity Q-TOF Mass Spectrometer and an Extended Tandem Mass Spectrum Library



Assignment of fragments observed in authentic standards.

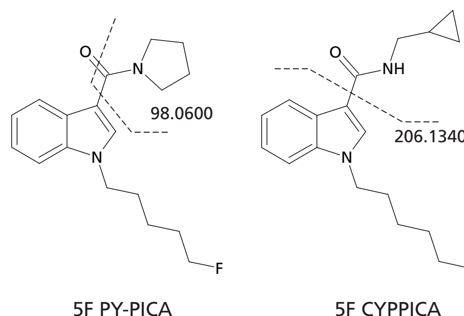


Figure 7 Library search results of Peak B. The match to 5-fluoro PY-PICA can be differentiated from 5-fluoro CYPPICA using the product ion spectrum. It should be noted that either fragment could in theory be predicted from either compound, however the authentic standard shows which fragments are actually formed. This demonstrates the importance of the library spectra for compound identification.

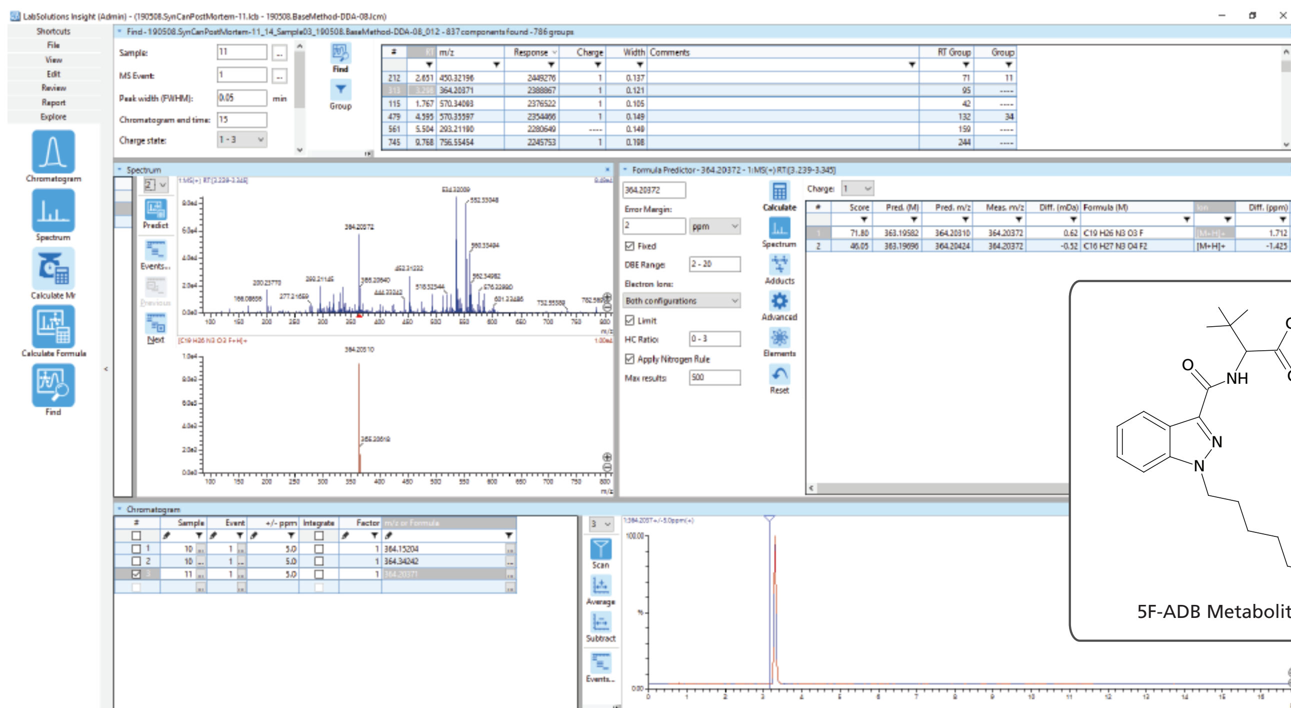
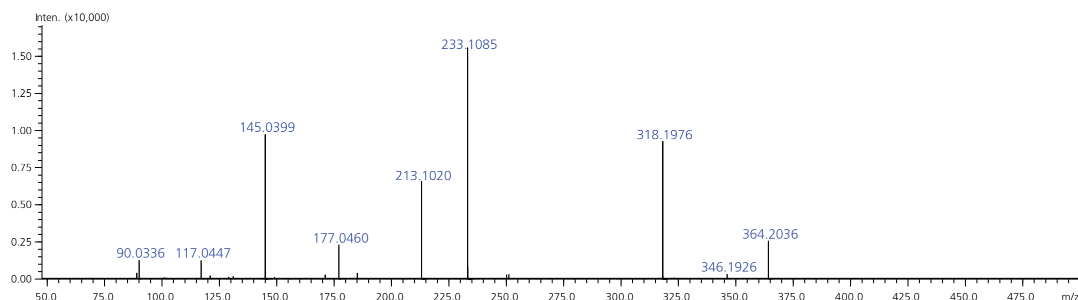
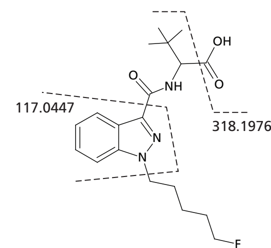


Figure 8 Non-targeted analysis using Insight Explore's 'Find' function.





Assignment of distinguishing fragment ions

Figure 9 Formula prediction was done on the feature at *m/z* 364.20371, which matched to 910 hits in a Chem-spider search. The data-dependent MS2 spectrum showed the presence of an indazole core group, narrowing the number of hits to just 5 structures. The only structure consistent with the full MS2 pattern was 5F-ADB Metabolite 7.

Conclusion

A new high-sensitivity LC-MS/MS method with an enhanced spectrum library was developed to detect and identify designer drugs. In a group of ten post-mortem blood samples, several cannabinoids were detected and identified based on RT and MS2 similarity to the library. Additionally, non-targeted analysis was used to detect a designer cannabinoid metabolite on the basis of accurate mass and tandem MS.

The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures. Not available in China.

First Edition: July, 2019

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedures.
This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Company names, products/service names and logos used in this publication are trademarks and trade names of Shimadzu Corporation, its subsidiaries or its affiliates, whether or not they are used with trademark symbol “TM” or “®”.
Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol “TM” or “®”.
Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2019