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Accurate Mass LC-MS/MS Profiling of Synthetic Cannabinoids

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Introduction

New synthetic designer drugs of abuse are being produced as readily available consumer products such as bath salts and incense. The uncontrolled and unregulated synthesis of these drugs causes numerous variations in formula, structure, and stereochemistry. These variations cause the analysis of the compounds of interest to be very difficult and extremely time consuming. In this study we describe a new simple and efficient method by which these new synthetic drugs of abuse can be rapidly identified and quantified.

Materials and Method

The samples received were herbal in nature. It was decided that aliquots of each sample would be extracted in acidic aqueous, basic aqueous and various organic solvents. The resulting extracts were then analyzed by LC-MS.

Sample Preparation

For each extraction 224 – 248 mg of sample was weighed into a 20 mL scintillation vial, 5 mL of extraction solvent was added, samples were vortexed and sconicated for 60 minutes. Following sonication the extraction solvent was removed to another vial and (with the exception of aqueous solvents) evaporated to dryness under nitrogen. Solvents used for extraction were as follows: $CHCl_3$, Acetone, IPA, MeOH, ACN, 0.1 % NH_4OH in water and 0.1 % formic acid in water. For LC-MS analysis samples were reconstituted with the addition of 2 mL of 0.1% formic acid in water followed by 3 mL of acetonitrile. Following solvent addition samples were vortexed, sonicated and analyzed by LC-MS. Aqueous extracts were analyzed as is.

Chromatography

Instrument   : Shimadzu XR system
Column   : Supelco Ascentis Express C-18 150 mm × 2.1 mm, 2.7 µm
Mobile Phase   : A: 0.1% Formic Acid in Water
                     B: 0.1% Formic Acid in ACN
Flow Rate   : 0.4 mL/min
Injection Volume : 25 µL

Gradient Program:

Gradient A			Gradient B	
Time (min)	%B		Time (min)	%B
0	5		0	30
35	20		45	90
49	90		54	90
54	90		55	30
55	5		60	30
60	5			

Mass Spectrometry

Instrument : Shimadzu LCMS-IT-ToF Mass Spectrometer
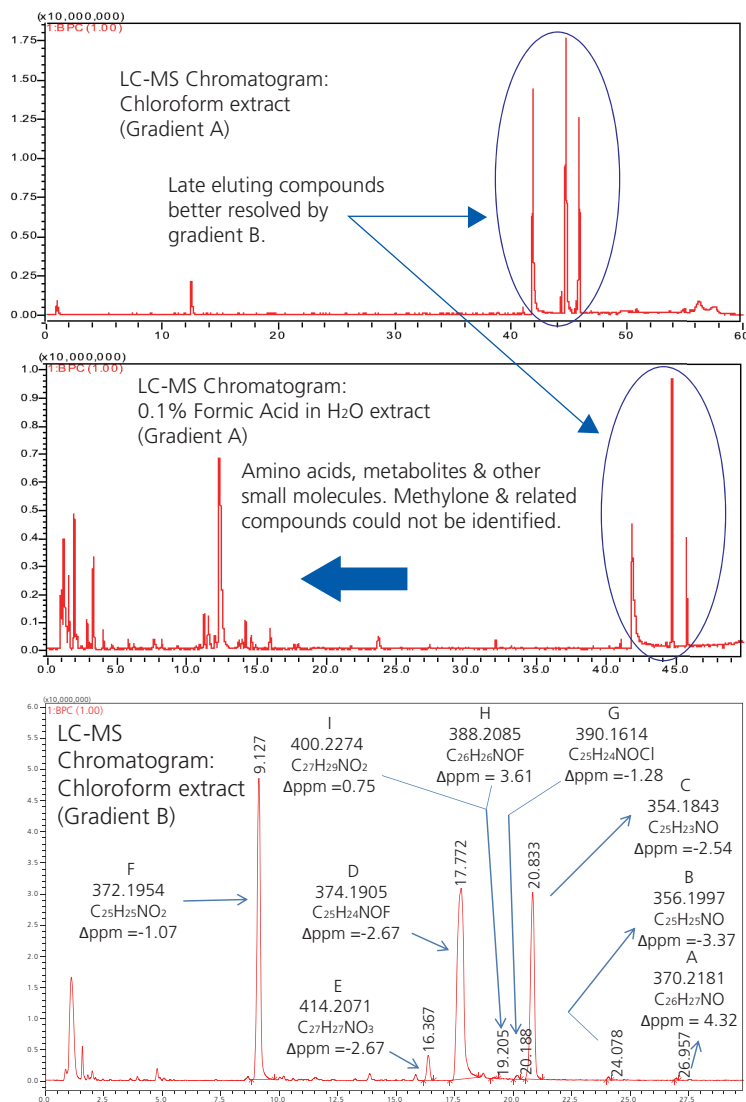Ionization : ESI
Polarity : Positive
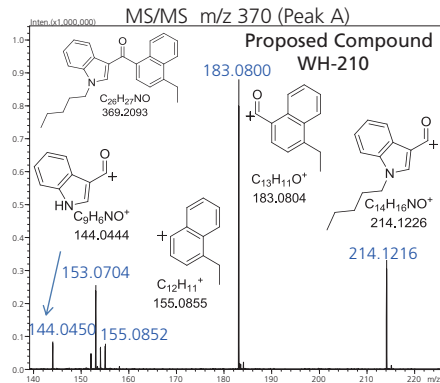Mass Range: 100 – 500 m/z

Data Analysis

ESI-MS accurate mass data was used to predict chemical formulas which were searched in databases such as ChemSpider (http://www.chemspider.com/) and MassBank (http://www.massbank.jp).
ESI-MS accurate mass data and MS/MS data were also searched using METLIN (http://metlin.scripps.edu/). MassFrontier 5.0 (ThermoFisher) was used to help evaluate MS/MS data and propose chemical structures.
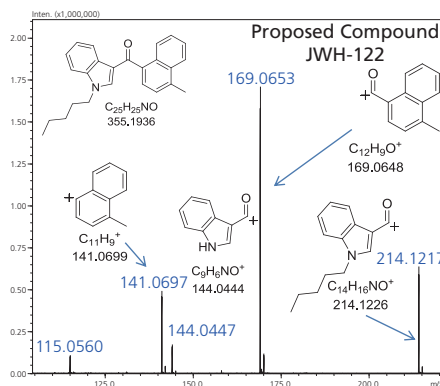
Results

LC-MS Chromatogram: Chloroform extract (Gradient A)

Late eluting compounds better resolved by gradient B.

LC-MS Chromatogram: 0.1% Formic Acid in H_2O extract (Gradient A)

Amino acids, metabolites & other small molecules. Methylone & related compounds could not be identified.

LC-MS Chromatogram: Chloroform extract (Gradient B)

F
372.1954
$C_{25}H_{25}NO_2$
Δppm =-1.07

D
374.1905
$C_{25}H_{24}NOF$
Δppm =-2.67

E
414.2071
$C_{27}H_{27}NO_3$
Δppm =-2.67

I
400.2274
$C_{27}H_{29}NO_2$
Δppm =0.75

H
388.2085
$C_{26}H_{26}NOF$
Δppm = 3.61

G
390.1614
$C_{25}H_{24}NOCl$
Δppm =-1.28

C
354.1843
$C_{25}H_{23}NO$
Δppm =-2.54

B
356.1997
$C_{25}H_{25}NO$
Δppm =-3.37

A
370.2181
$C_{26}H_{27}NO$
Δppm = 4.32

9.127
17.772
20.833
16.367
19.205
20.188
24.078
26.957

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MS/MS m/z 370 (Peak A)

Proposed Compound WH-210

$C_{26}H_{27}NO$ 369.2093

183.0800

$C_{13}H_{11}O^+$ 183.0804

$C_{14}H_{16}NO^+$ 214.1226

214.1216

$C_9H_6NO^+$ 144.0444

$C_{12}H_{11}^+$ 155.0855

153.0704

144.0450 155.0852

Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
183.0800	0	100.0	10, 20, 40	[M+H]+	
214.1220	1	33.1	10, 20, 40	[M+H]+	
155.0850	1	73.1	20, 40	[M]+	
144.0440	6	38.4	20, 40	[M+H]+	
153.0690	9	35.3	20, 40	-	No Structure Information is available

METLIN ID: **64778** NAME: **JWH 210** MASS: **369.2093** View MS/MS STRUCTURE

METLIN: Fragment Ion Metabolite Search
LC Peak A: Proposed Match JWH-210

Proposed Compound JWH-122

$C_{25}H_{25}NO$ 355.1936

169.0653

$C_{12}H_9O^+$ 169.0648

$C_{11}H_9^+$ 141.0699

$C_9H_6NO^+$ 144.0444

$C_{14}H_{16}NO^+$ 214.1226

214.1217

141.0697 144.0447

115.0560

Frag. m/z	Δppm	Intensity	CE	Predicted Ion Type	Predicted Fragment Structure
141.0690	4	100.0	10, 20, 40	[M]+	
214.1200	7	29.1	10, 20, 40	[M+H]+	
169.0630	13	100.0	10, 20, 40	[M-H+2H]+	
115.0540	17	14.0	20, 40	[M+H]+	

METLIN ID: **64771** NAME: **JWH 122** MASS: **355.1936** View MS/MS STRUCTURE

METLIN: Fragment Ion Metabolite Search
LC Peak B: Proposed Match JWH-122

LC Peaks A & B: The proposed structures for LC peaks A and B are known ethyl and methyl napthyl analogues of synthetic cannabinoid JWH-018. The structures for these two compounds were proposed based on the ability to match accurate mass and MS/MS data with an existing entry in the METLIN database. The ability to match structures A and B with existing database entries greatly expedited the process of identifying other similar compounds present in the herbal sample extract.

$C_{25}H_{23}NO$ 353.1780

169.0647

★ Proposed structure double bond position not determined.

Based on the observed accurate mass and MS/MS data two structures wereproposed for m/z 354.1843

141.0696 144.0441

$C_{10}H_8NO^+$ 158.0600

$C_{14}H_{14}NO^+$ 212.1070

212.1056

115.0542 158.0600
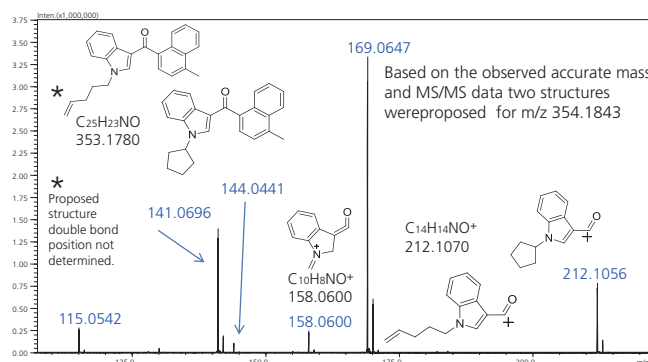
LC Peak C: The MS/MS data for LC Peak C suggested that the compound was a methyl napthyl compound similar to LC peak B (JWH-122). The MS/MS data further suggested that the difference between compounds B and C was the addition of a double bond or ring (∆ = 2 Da) to the nitrogen R group. A ChemSpider similarity search of the two structures yielded one compound with a chemical formula of $C_{25}H_{23}NO$ (JWH-150). MS/MS data did not match this structure, however a third structure could be proposed based on this.

MS/MS *m/z* 374 (Peak D)

MS Spectrum Peak G

MS/MS *m/z* 390 (Peak G)
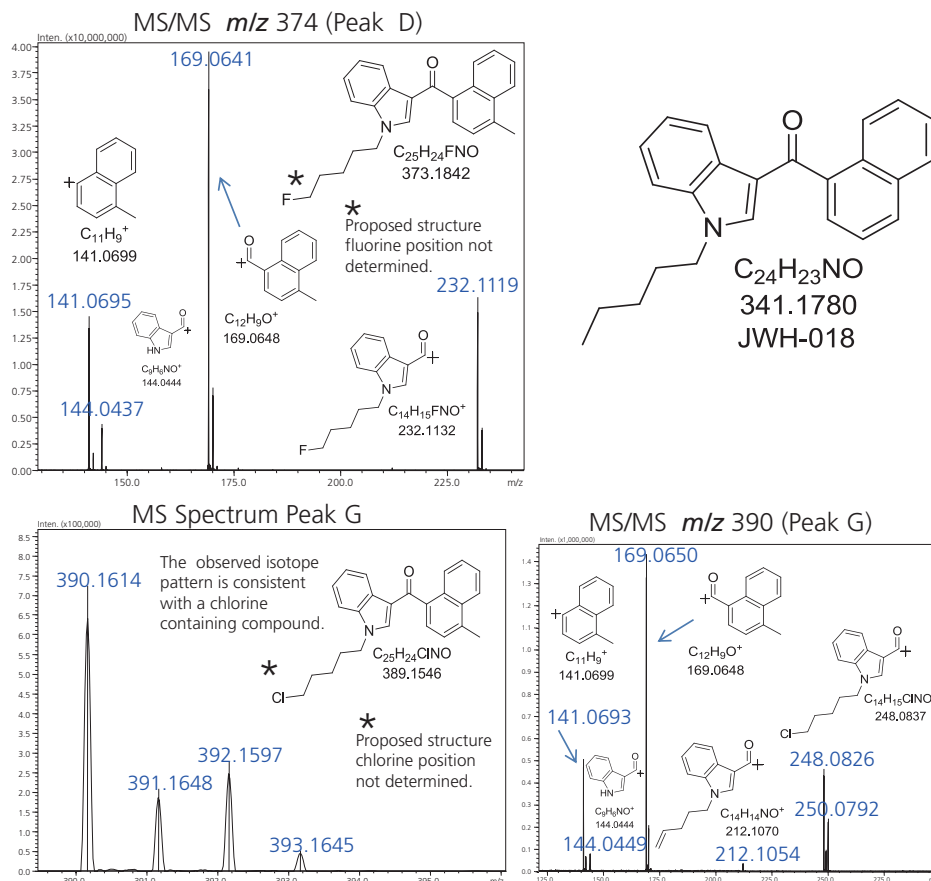
LC Peaks D & G: The proposed structures for compounds D & G are structurally similar to known halogenated analogues of synthetic cannabinoid JWH-018.

Conclusion

From the herbal sample chloroform extract 13 observed compounds had accurate mass and MS/MS data consistent with synthetic cannabinoids.

All compounds gave either a *m/z* 169 or *m/z* 183 fragment ion, which suggests that they are either methyl or ethyl napthyl analogues to JWH-018. Proposed chemical formulas also suggest that some compounds are halogenated.

Of the compounds observed two (JWH-210 & JWH-122) were identified using the METLIN metabolite data base accurate mass & MS/MS ion search. These two compounds were "certified" by the vendor to not be present in this product. It should be noted that this study was not quantitative in nature, and information regarding vendor method detection limits were not available. JWH-210 & JWH 122 may be present at levels less than the PDL of the vendor's method.

From a detection stand point; the results indicate that LC-MS/MS precursor ion scanning on a QqQ or QqToF may be a viable option to screen for classes of synthetic cannabinoids.

Acknowledgements

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