Automated Ion Trap Library Screening Method for the Detection of Synthetic Cannabinoids in Commercial Herbal Incense Products

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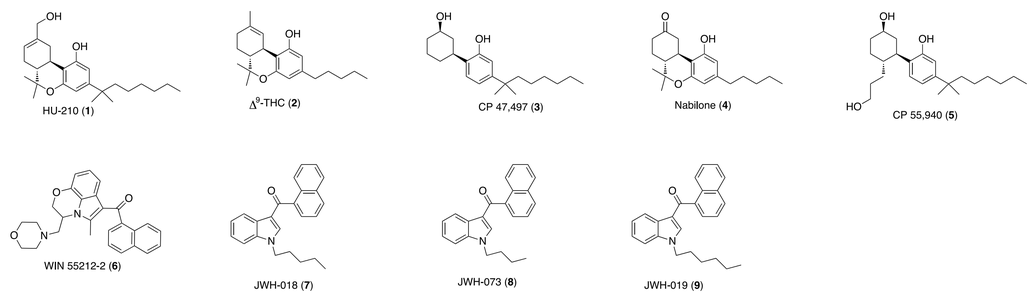
BACKGROUND

In 2008, a German pharmaceutical company by the name of THC Pharm confirmed the presence of a synthetic cannabinoid in a commercial herbal incense product called 'Spice'.[1] It was believed at the time that commercial incense products were being sprayed with these synthetic cannabinoids and used as designer marijuana substitutes. Shortly after this report, seven more incense blends were tested by researchers at the Institute of Forensic Medicine at the University Medical Center Freiburg and were confirmed to contain 5 different known synthetic cannabinoids.[2] In North America, the first report of synthetic cannabinoids being positively identified in incense products was made by the Customs and Border Protection – Chicago Laboratory.[3] Five incense products were tested by GC/MS and found to contain small but verifiable amounts of HU-210 (1), a known cannabinoid with a potency much greater than Δ^9-tetrahydrocannbinol (THC) (2).[4,5] In another instance, the Wisconsin State Crime Laboratory identified an unknown white powder, suspected to be cocaine or methamphetamine, as the synthetic cannabinoid CP47,497 (3).[6] Recently, Goda *et al* have identified synthetic cannabinoids in 44 of 46 herbal incense products. In an effort to determine the scope of this new designer drug phenomenon our lab has prepared several synthetic cannabinoids standards and developed an automated method to screen for and positively identify these compounds in commercial herbal incense products.

Figure 1 Classical and Non-Classical Cannabinoids



HPLC-MS^n CONDITIONS

Agilent 1100 HPLC
Mobile Phase A: 90:10:10mM
H_2O/MeCN/NH_4OAc
Mobile Phase B: 90:10:10mM
MeCN/H_2O/NH_4OAc

Gradient:
0-20 min 50-90%B
20-25 min 90%B
25.1-32 min 50%B
4.6x250mm, 5µm Luna Phenyl-Hexyl
1mL/min
30°C
UV @ 225nm

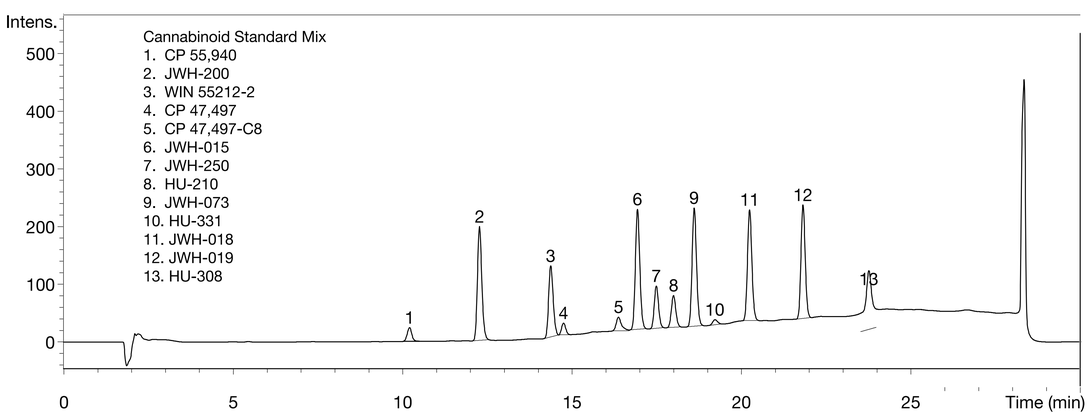
Agilent G2445A MSD Trap MS with Bruker Trap Control SW v4.1
+ESI Conditions
Nebulizer 70 psi
Drying gas Flow 12 L/min
Drying gas temperature 325°C
ICC target 30000
Max Accum. Time 300ms
Scan range 100-800 m/z

Data Dependent MS/MS Parameters
Auto MS^2
SmartFrag ON
Frag Amplitude 1.5V
Amplitude Ramp 30-200%
Include Masses (319.10, 328.20, 329.20, 333.20, 336.20, 342.20, 356.20, 359.30, 385.20 387.30, 397.30, 399.20, 415.30, 427.20)
m/z Isolation Width 4m/z

Library Search Parameters
S/N = 3
Ret time window 0.4 min
Intensity Threshold 1000000
Desired Score = 900
Minimum Score = 300

(Library Score is a 0-1000 scale calculation of the closeness of fit of the observed spectra to the library spectra. Values from 900-1000 are considered a very good fit, 600-900 are considered a good fit, and values <600 are considered a poor fit.)

Figure 2 Optimized Chromatography for 13 Synthetic Cannabinoid Standards



RESULTS

The successful use of MSn libraries for the screening and identification of large numbers of target analytes has been demonstrated previously.[8] In this approach a standardized HPLC method is used in order to match retention times with known standards and both MS and MS/MS spectra are generated under reproducible conditions and imported into a searchable mass spectral library database. It is important that the MS/MS spectra are reproducible as the spectral matching algorithm used for the library search compares the ions of the mass spectra as well as their relative intensities. Ion Trap MS is ideally suited for this application because fragmentation occurs in discrete stages and can be carefully controlled at each stage. The steps involve isolation of a target m/z, applying a tunable Fragmentation Amplitude voltage which very selectively fragments only the isolated mass, followed by detection of the resulting fragment ion mass spectrum. Positive identification requires that the retention time, MS, and MS/MS spectra all match within predefined limits.

Figure 3 Herbal Incense Blends Tested

Herbal Incense products were purchased from a variety of commercial sources between 01-01-2010 and 02-01-2010.

Table 1 Commercially Available Synthetic Cannabinoids and Major Ions Observed in MS and MS/MS Library Spectra

	Cannabinoid	Catalog No.	Ret. Time (min)	Major MS Ion(s) (amu)	Major MS/MS Ions (amu)
1	(±)-CP 55,940	13241	10.2	415.2, 399.3, 359.3, 233.1	341.1, 233.1, 215.0, 147.0, 121.0
2	JWH-200	13171	12.3	385.2	155.0, 145.0, 117.1
3	WIN 55212-2	10009023	14.4	427.2	155.0, 145.0, 127.0
4	(±)-CP 47,497	16851	14.7	488.3*, 319.2, 301.2, 279.1	301.2, 233.1, 175.0, 107.1
5	(±)-CP 47,497-C8	13216	16.4	488.3*, 355.3, 333.3, 315.3,175.1	315.3, 247.2, 175.0, 107.1
6	JWH-015	10009018	16.9	328.2	200.0, 155.0, 145.0
7	JWH-250	13634	17.5	336.2	188.1, 121.1
8	HU-210*	90082	18	488.3*, 409.3, 387.3,	369.2, 261.1, 243.1, 201.0, 189.0
9	JWH-073	13170	18.6	328.2	200.0, 155.0, 145.0, 117.0
10	HU-331	10005673	19.2	488.3*, 351.2, 329.2, 311.2	311.2, 287.1, 273.1, 259.1, 209.1
11	JWH-018	13169	20.2	342.2	214.1, 155.0, 145.0, 117.1
12	JWH-019	13633	21.8	356.2	228.1, 155.0, 145.0, 117.1
13	HU-308	90086	23.7	488.3*, 447.2, 437.2, 415.3, 397.3	355.2, 303.2, 277.2, 271.1, 215.0, 133.0

*(DEA Schedule 1 Regulated)

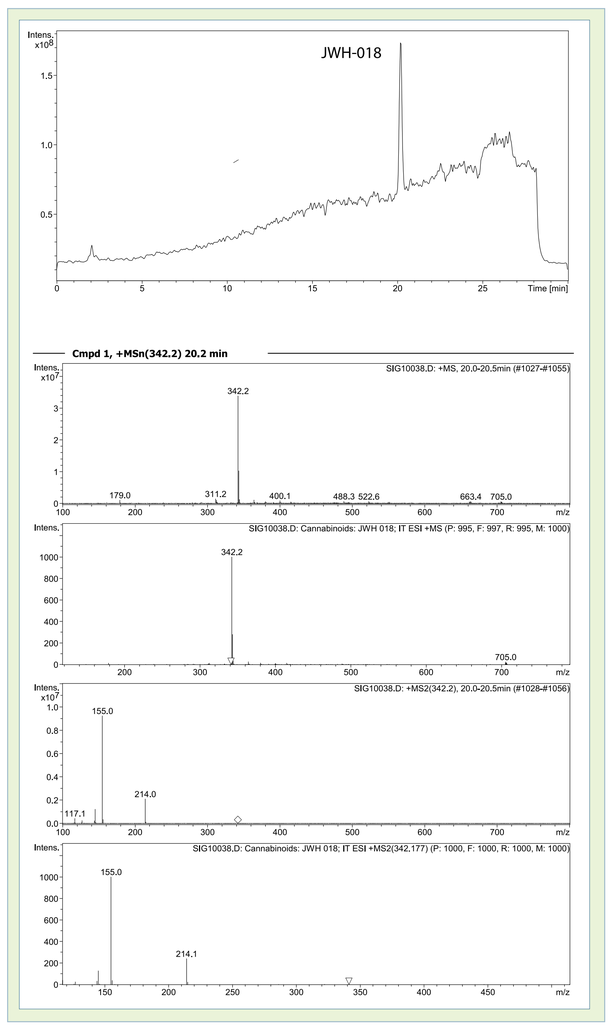
Table 2 Library Search Results

Cannabinoid	Spice	K2	Tribal Warrior	Spike99	ExSES	Neder Gold
(±)-CP 55,940						
JWH-200						
WIN 55212-2						
(±)-CP 47,497					manual detect*	
(±)-CP 47,497-C8				746*	691*	
JWH-015						
JWH-250			947	991		
HU-210						
JWH-073		912	975			
HU-331						
JWH-018	999	922	938	912		
JWH-019				982		
HU-308						

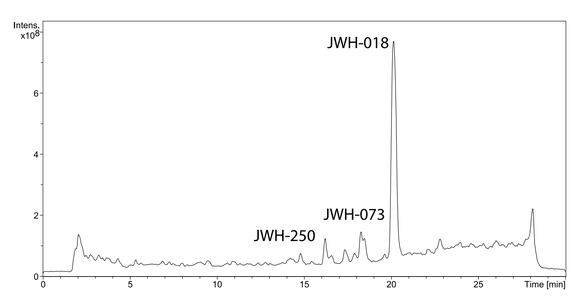
* The suspected trans isomers, which are known byproducts in the syntheses of these compounds, were also detected.

Figure 4 Herbal Incense Positive Hits

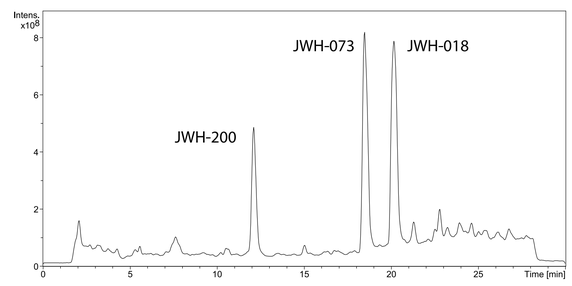
Spice



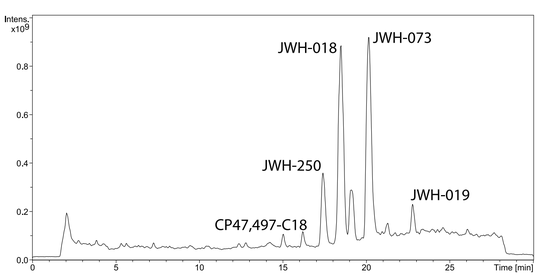
Tribal Warrior



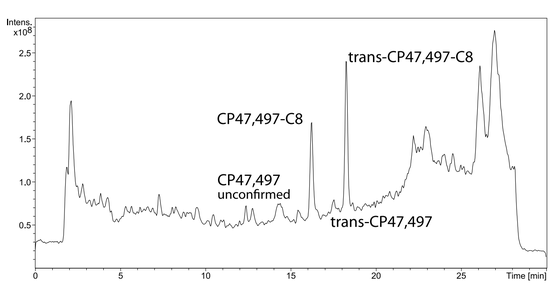
K2



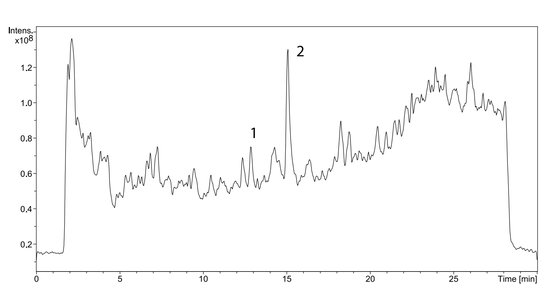
Spike 99



ExSES



Neder Gold



CONCLUSION

An ion trap MS/MS Library of 13 synthetic cannabinoids was developed and used to positively identify the compounds in commercially available herbal incense. By this approach, 5 of the 6 products were found to contain synthetic cannabinoids. Synthetic cannabinoids detected include JWH-018, JWH-073, JWH-019, JWH-250, CP 47,497, and CP 47-497-C8 homolog.

References

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