

# Analysis of Synthetic Cannabinoids in Herbal Blends by GC Tandem Mass Spectrometry

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## Introduction

Synthetic cannabinoids represent a new and growing list of scheduled drugs. As these newly introduced compounds become regulated, there is a need for confirmatory analysis. However, obtaining reference standards can be problematic and libraries of mass spectra can be difficult to come by. Unlike other controlled substances, the matrix is not a "pure" drug or simple pill/powder. The botanical matrix that acts as a substrate creates significant data interpretation challenges and therefore a library of mass spectra for GC Gas Chromatography (GC) single quadrupole was developed and validated. To aid with data interpretation an automated Deconvolution Reporting Software (DRS) library was developed. This tool uses a mathematical algorithm to extract spectra, search potential components against a known spectral library and generate a quantitative report for the matched compounds in the sample. An alternative approach is to isolate the targets of interest via tandem mass spectrometry. The process of multiple reaction monitoring (MRM) offers high specificity for each analyte and lower detection limits in a complex matrix. The data from tandem mass spectrometry is significantly easier to interpret and offers higher confidence in the results.

## Experimental

JWH-018, JWH-073, JWH-200, JWH-250, CP-47,497 (C7 analogue) and HU-210 were prepared as 5 µg/mL stock solutions. The matrix was prepared by grinding approximately 500 mg of material between two 5 in. x 5 in. sheets of sandpaper until a finely divided powder was developed. Both acidic and basic liquid-liquid extractions were employed. Derivatization was achieved via commercially available BSTFA in ethyl acetate. Specific MRM transitions and collision energies were determined through a series of experiments. GC-tandem mass spectrometry facilitated chromatographic resolution and molecular specificity required to identify and quantify each of the analytes.

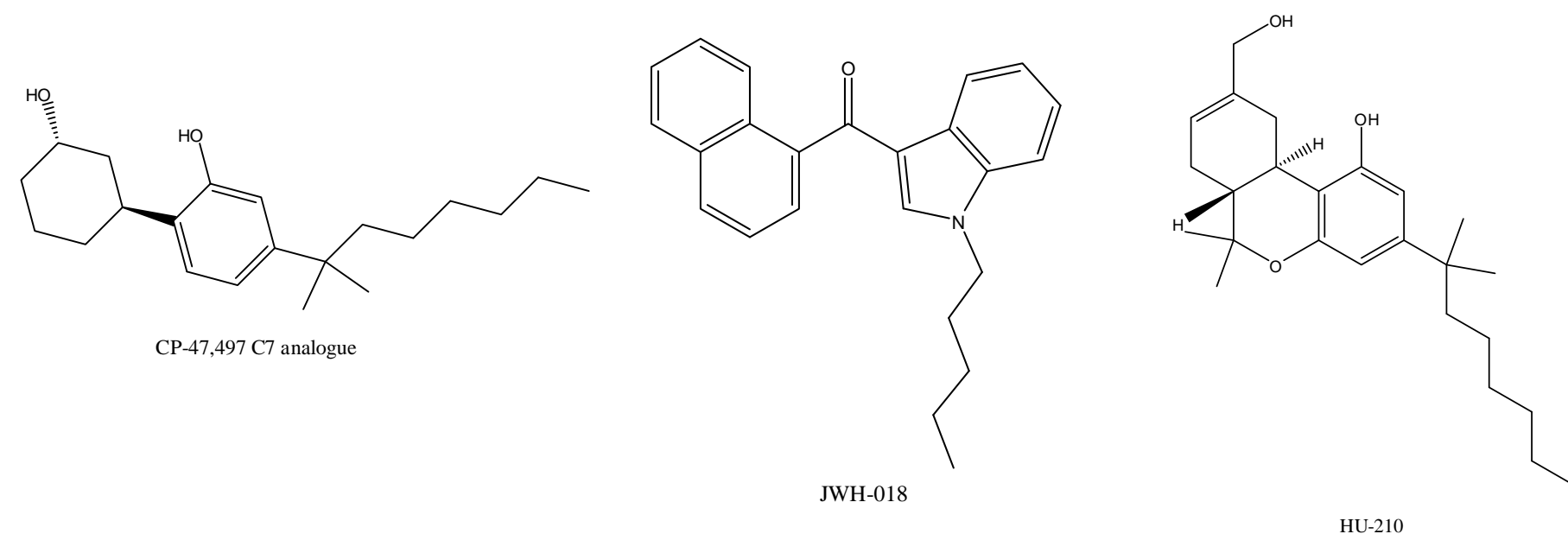
## Experimental

GC Run Conditions	
Column 1	HP-5MS UI (Agilent Santa Clara, CA)
Injection mode	Pulsed split-less
Inlet temperature	300 °C
Injection volume	1 microliter
Carrier gas	Helium, constant flow mode, 1.2 ml/min
Oven program	80 °C (hold 0.17 min) then 30 °C/min to 300 °C (hold 0.5 min) then 5 °C/min to 340 °C (hold 5 min)
Transfer line temperature	325 °C
MS conditions	
Tune	Autotune
Gain factor	50
Acquisition parameters	Electron Impact, multiple reaction monitoring
Collision gas	Nitrogen, 1.5 ml/min; Helium quench gas 2.25 ml/min
Solvent delay	7.0 minutes
MS temperatures	Source 300 °C, Quadrupoles 150 °C

### MRM Transitions and collision energies

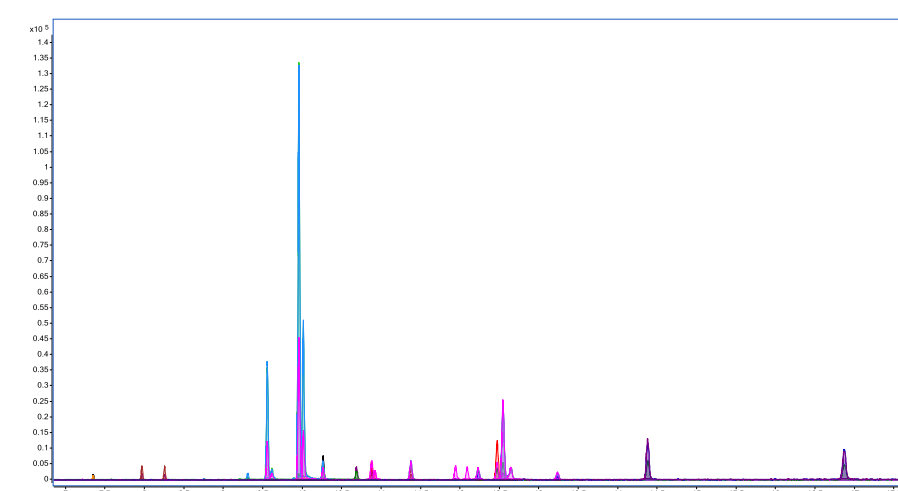
Compound Name	Precursor Ion	Product Ion	Collision Energy
AM-694	435	232	27
AM-694	435	220	13
CP-47-497-C8	377	191	29
CP-47-497-C8	377	167	33
HU-211	530	446	13
HU-211	446	299	21
JWH-015	327	310	10
JWH-015	310	268	23
JWH-018	341	167	23
JWH-018	324	254	23
JWH-073	327	167	23
JWH-073	310	254	23
JWH-081	371	197	23
JWH-081	354	269	31
JWH-122	338	268	23
JWH-122	298	181	12
JWH-133	312	269	12
JWH-133	269	93	23
JWH-200	384	100	23
JWH-200	100	56	17
JWH-203	339	214	3
JWH-203	214	144	17
JWH-250	335	214	3
JWH-250	214	144	17
JWH-251	214	144	17
JWH-251	144	116	12
JWH-398	375	201	23
JWH-398	318	189	23
RCS-4	321	264	19
RCS-4	264	135	17
RCS-8	254	158	13
RCS-8	254	144	19
WIN55 212-3/2	100	70	13
WIN55 212-3/2	100	56	15

## Results and Discussion



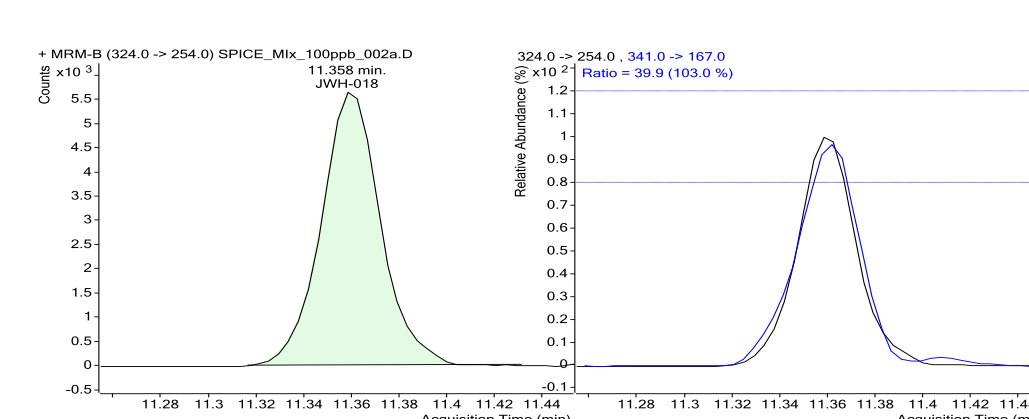
Synthetic cannabinoids fall into the three structural categories as shown above. From right to left, one class possesses a structural scaffold similar to that of tetrahydrocannabinol (THC). The second type are synthetic naphthoylindole analogues and finally, the third type posses a phenylcyclohexyl moiety. As is true for THC, a common motif inherent to most synthetic cannabinoids is a short aliphatic chain known to interact with the cannabinoid CB1 and CB2 receptors.

### MRM TIC 100 ng/ml standard mix



MRM total ion chromatogram for 100 ng/mL of the standard mixture. All 17 synthetic cannabinoid standards were easily found.

### Example Compound: JWH-018, 100 ng/ml



The ratio of the qualifying ion to that of the quantifier is used to further confirm positive identification of each analyte in matrix in addition to retention time information and precursor / product ion pairings. In this preliminary study, calibration curves were prepared over the range of 5 to 500 ng/mL and linearity was observed over this range.



The herbal blends analyzed in this study were: EX 565, K2 Blondie, K4 Purple Haze, K3 XXX, Lunar Diamond, Zombie, and K2 Diamond.

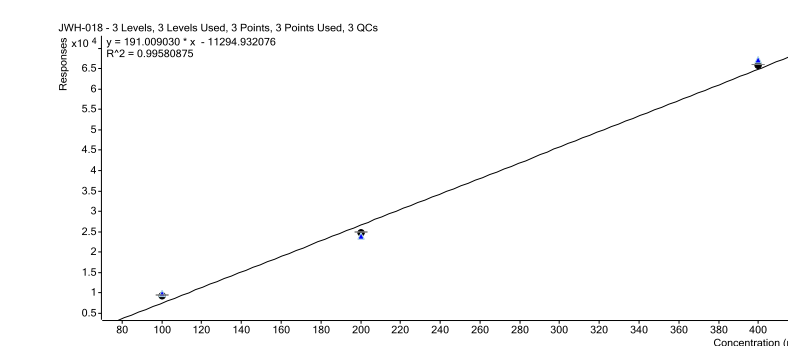
Lack of bulk sample homogeneity require some degree of mixing to ensure representative samples are tested.

- Matrix difficult to homogenize
- Mortar and pestle
- Hand-held herb grinder
- Electrical devices
- Sandpaper



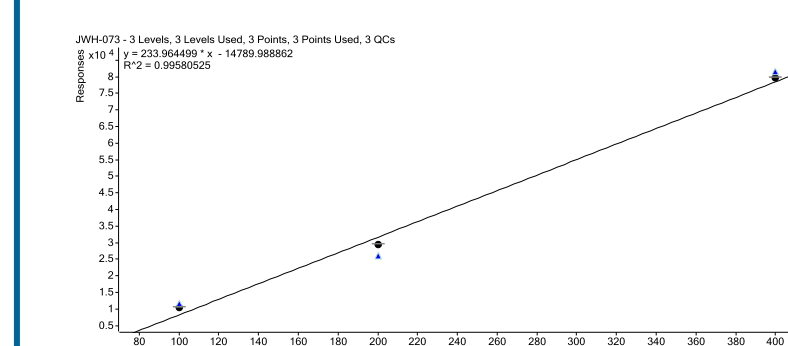
## Results and Discussion

### Cal Curve, JWH-018 100ng/ml – 400 ng/ml



Black dots = Calibrators  
Blue triangles = QC's  
R<sup>2</sup> = 0.996  
Externally standardized

### Cal Curve, JWH-073 100ng/ml – 400 ng/ml



Black dots = Calibrators  
Blue triangles = QC's  
R<sup>2</sup> = 0.996  
Externally standardized

Calibration curves were then constructed over the range of 100 – 400 ng/ml and replicate injections (n = 3) were made at each level. The average correlation coefficient of linearity for the all analytes in this study (r2) was 0.99 +/-0.012. The precision, measured as %RSD, was 13%, 7%, and 6% at 100 ppb, 200 ppb, and 400 ppb, respectively and levels of quantification as determined by a signal to noise ratio >= 10, were determined to range from 1 – 100 ng/ml in the heavy botanical matrix.

All of the blends analyzed contained two or more synthetic cannabinoids and several had as many as five different components. This was confirmed by the correct ratio of the qualifying ion to that of the quantifying ion and retention time accuracy JWH-073 and JWH-018 were detected in all of the blends analyzed at concentrations ranging from 50 to 150 ppb. Notably, K2 Blondie contained JWH-073 and JWH-018 at concentrations extrapolated to be as much as 1,000-fold higher than the other blends.

Herein, representative samples of synthetic cannabinoid herbal blends were analyzed to demonstrate the applicability of a GC/MS/MS analytical approach. Compared to selected ion monitoring (SIM), multiple reaction monitoring (MRM) made possible by GC/MS/MS systems offers significantly improved selectivity and sensitivity for the detection of trace-level synthetic cannabinoids complex matrices such as herbal incense blends. Furthermore, GC/MS/MS eliminates the need for post acquisition mass spectral deconvolution with subsequent library searching for identification and quantification. The utility of triple quadrupole MS cannot be overstated. Its ability to mitigate matrix effects and improve signal-to-noise significantly increases the analysts confidence.

## Conclusions

Multiple reaction monitoring in GC tandem mass spectrometry offers a unique advantage over single quadrupole selected ion monitoring (SIM) in its ability to differentiate interfering matrix components from that of the analyte. The probability of an interfering ion having the same product ion spectrum is low, thus differentiation of matrix and analyte can be achieved. Through GC tandem mass spectrometry, highly selective and sensitive analytical methods in complex matrices can be developed, assuring positive identification and lowering detection limits far beyond those achieved in single quadrupole mass spectrometry. In this preliminary study, a selective and sensitive analytical method for the analysis of six common synthetic cannabinoids was developed.

## Bibliography

Macherone A, Gluodenis TJ. Confirmation and Quantification of Synthetic Cannabinoids in Herbal Incense Blends by Gas Chromatography-Tandem Quadrupole Mass Spectrometry (GC/MS/MS). Agilent Application Note 5990-8987EN (2011)

Macherone A. Synthetic Cannabinoids: the Analytical Challenges. Agilent Live Webinar August 10, 2011