Confirmation and Quantification of Synthetic Cannabinoids in Herbal Incense Blends by Triple Quadrupole GC/MS

Application Note

Forensic Toxicology

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Abstract

With the rapid growth in popularity of herbal incense blends containing synthetic cannabinoids, today's forensic laboratories are challenged to confirmandquantify the controlled forms at trace levels in complex matrices with confidence.Here,a representative sample of 17 of the more than 30 known synthetic cannabinoids is analyzed to demonstrate the applicability of a Triple Quadrupole GC/MS analytical method. The method's selectivity reduces matrix effects and improves signal-to-noise, significantlyincreasingconfi denceinanalyticalresults.Themethodalso eliminates the need for post data-acquisition processing such as mass spectral deconvolution.

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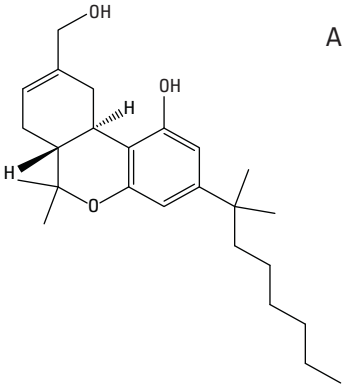
Introduction

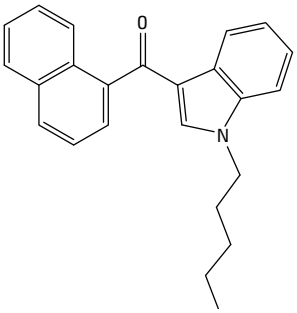
Synthetic cannabinoids are cannabinomimetic compounds originally synthesized for medical research. The rapid growth in use of these compounds is of serious concern in many countries including the U.S.

Synthetic cannabinoids fall into the three structural types shown in Figure 1. The firsttype(1A)possessesastructural scaffold similar to that of tetradydrocannabinol. The second type (1B) is synthetic napthoylindole analogues. The third type (1C) is phenylcyclohexyl moieties. A common motif inherent to most synthetic cannabinoids is a short aliphatic chain known to interact with the cannabinoid CB1 and CB2 receptors.



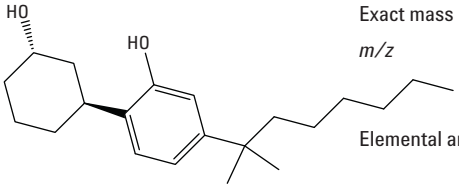
A **HU-210**

Chemical formula	$C_{25}H_{38}O_3$
Exact mass	386.2821
m/z	386.28 (100%) 387.29 (27.6%) 388.29 (4.3%)
Elemental analysis	C 77.68 H 9.91 O 12.42



B **JWH-018**

Chemical formula	$C_{24}H_{23}NO$
Exact mass	341.1780
m/z	341.18 (100%) 342.18 (26.3%) 343.18 (3.5%)



C **CP-47, 497 (C7) analogue**

Chemical formula	$C_{21}H_{34}O_2$
Exact mass	318.2559
m/z	318.26 (100%) 319.26 (23.2%) 320.26 (2.9%)
Elemental analysis	C 79.19 H 10.76 O 10.05

Figure 1. Synthetic cannabinoids fall into the three distinct structural patterns.

Synthetic cannabinoids are usually formulated in botanical matrices (Figure 2) and marketed for sale as herbal incense. Because they are labeled as *not for human consumption*, there is no oversight by the U.S. Food and Drug Administration (FDA). As such, there is no control over their manufacture, raw material quality, potency, and overall safety.

Figure 2. Synthetic cannabinoids are often formulated in botanical matrices.

Though many countries, including the U.S., have banned specificformsofthesecompounds,thelargeandgrowing number of synthetic cannabinoids has impeded their control. As soon as legislation is passed banning use of a specific form, a new one is synthesized and introduced. Due to the health risks and public threat associated with their use, the U.S Drug Enforcement Administration (DEA) exer-cised its emergency authority to control fivespecifi csyn-thetic cannabinoids for at least one year while it and the U.S. Department of Health and Human Services (DHHS) determine whether permanent control is warranted [1,2]. The DEA now controls:

- JWH-018
- JWH-073
- JWH-200
- CP-47,497 (C7)
- CP-47,497 (C8)

HU-210 is controlled under a previous DEA ruling. Over 20 uncontrolled forms remain and the number is growing.

Confirmation and quantification of synthetic cannabinoid analogs and homologs by single quadrupole gas chromatography/mass spectrometry (GC/MS) presents numerous analytical challenges. At the outset, the botanical matrix is surprisingly difficult to homogenize. Subsequent extraction requires a general approach because synthetic cannabinoids contain a variety of functional groups. However, a general approach extracts a large amount of matrix substances which in turn produce a complex chromatogram with a substantial number of peaks.

The blends often contain a mixture of synthetic cannabinoids which, due to their structural similarities and isomeric forms, co-elute producing overlapped mass spectra. Adding to the challenge, synthetic cannabinoids can be extremely potent and thus present at trace levels relative to the matrix. Though previously demonstrated as an effective and easy to replicate approach [3, 4], single quadrupole GC/MS analyses of these matrices yields very complex data that requires significant effort to interpret without the help of special post acquisition processing software, for example mass spectral deconvolution software.

In this application, a representative sample of an herbal blend is analyzed for the presence of synthetic cannabinoids to demonstrate the applicability of an alternative GC/MS/MS approach that offers enhanced selectivity and sensitivity, and that eliminates the need for mass spectral deconvolution.

Experimental

Reference standards and samples

Listed in Table 3, seventeen of the more than 30 known synthetic cannabinoids were chosen for the development of the GC/MS/MS analytical method. These compounds were chosen to capture the structural diversity of synthetic cannabinoids found in popular herbal blends.

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

Sample Preparation

Homogenization

The botanical material used as the carrier for synthetic cannabinoids, for example Damiana (*Tumera diffusa*), is soft and light. These properties make it difficult to crush into a homogenous form for representative sampling. For this analysis, approximately 500 mg of sample was ground between two 5 inch by 5 inch sheets of 100-grit sandpaper until a finely divided powder was obtained.

Extraction

The multiple functional groups associated with synthetic cannabinoids necessitate a generalized extraction approach. For this analysis, an acid/base combined extraction followed by centrifugation was employed. It is also possible to perform the extraction using methanol incubation. Either approach will extract substantial amounts of matrix components.

Using the acid/base approach, an aliquot of homogenized sample (50 – 100 mg) was acidified by adding 1 mL of de-ionized water, followed by three drops of 10% hydrochloric acid. Next, 1 mL of solvent (95% methylene chloride/5% isopropanol v/v) was added and the sample mixed. The sample was then centrifuged and the bottom solvent layer retained and set aside. Two drops of concentrated ammonium hydroxide and 1 mL of the solvent (95% methylene chloride/5% isopropanol v/v) were added to the remaining aqueous mixture (top layer). The sample was mixed and centrifuged again. The bottom solvent layer was removed, combined with the first bottom solvent layer collected, and then mixed briefly. The sample was then ready for GC/MS/MS analysis.

Derivatization

Some synthetic cannabinoids, for example HU-210, contain multiple, active, polar functional groups such as phenols and alcohols, which can make them much less amenable to GC/MS analysis. To enhance the chromatographic performance and sensitivity of the method for these compounds, derivatization with BSTFA (N,o-Bis (Trimethylsilyl) trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) can be used to cap the functional groups and to produce more intense ions for identification and quantification. Derivatization is not required for the analysis presented in this application note.

GC/MS/MS Analysis

The GC/MS/MS analyses were performed on an Agilent 7000 Series Triple Quadrupole GC/MS system which couples the Agilent 7890A Gas Chromatograph with the Agilent 7000B Mass Spectrometer.

The Agilent 7890A Gas Chromatograph was equipped with a HP-5MS UI column. Table 1 lists the Gas Chromatograph run conditions.

The Agilent 7000B Mass Spectrometer was operated in electron impact ionization (EI) MS/MS mode using multiple reaction monitoring (MRM) for all analytes and reference standards. Table 2 lists the Mass Spectrometer operating conditions.

Table 1. Gas Chromatograph Run Conditions

Agilent 7890A Gas Chromatograph run conditions	
Column 1	HP-5MS UI (Agilent Santa Clara, CA)
Injection mode	Pulsed split-less
Inlet temperature	300 °C
Injection volume	1 µL
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

Table 2. Mass Spectrometer Operating Conditions

Agilent 7000B Mass Spectrometer operating conditions	
Tune	Autotune
Gain factor	50
Acquisition parameters	Electron impact ionization, multiple reaction monitoring
Collision gas	Nitrogen, 1.5 mL/min Helium quench gas 2.25 mL/min
Solvent delay	7.0 min
MS temperatures	Source 300 °C, Quadrupoles 150 °C

MRM transitions were developed empirically beginning with the collection of full-scan spectra from the reference standards, followed by product ion scanning to identify optimal precursor/product ion pairs for the analysis. Next, the collision cell energy was optimized to achieve the maximum ion intensity for each unique transition. Table 3 provides the analyte list with the associated precursor and product ions, and the optimized collision energies.

Table 3. Analyte List with Associated Precursor and Product Ions, Optimized Collision Energies, and Retention Times

Compound name	Precursor ion	Product ion	Collision energy	Retention time (min)
AM-694	435	232	27	10.918
AM-694	435	220	13	
CP-47-497-C8	377	191	29	7.967
CP-47-497-C8	377	167	33	
HU-211	530	446	13	9.306
HU-211	446	299	21	
JWH-015	327	310	10	10.684
JWH-015	310	268	23	
JWH-018	341	167	23	11.375
JWH-018	324	254	23	
JWH-073	327	167	23	10.875
JWH-073	310	254	23	
JWH-081	371	197	23	13.238
JWH-081	354	269	31	
JWH-122	338	268	23	12.226
JWH-122	298	181	12	
JWH-133	312	269	12	7.348
JWH-133	269	93	23	
JWH-200	384	100	23	14.373
JWH-200	100	56	17	
JWH-203	339	214	3	9.954
JWH-203	214	144	17	
JWH-250	335	214	3	10.007
JWH-250	214	144	17	
JWH-251	214	144	17	9.553
JWH-251	144	116	12	
JWH-398	375	201	23	12.539
JWH-398	318	189	23	
RCS-4	321	264	19	10.259
RCS-4	264	135	17	
RCS-8	254	158	13	12.463
RCS-8	254	144	19	
WIN55 212-3/2	100	70	13	14.373
WIN55 212-3/2	100	56	15	

Results and Discussion

In a GC/MS/MS MRM experiment, the target analyte is selectively isolated from the matrix. As shown in Figure 3, the first quadrupole mass filter isolates a single precursor ion which is allowed to pass into the collision cell. In the collision cell, the precursor ion is fragmented by a collision gas and an applied electrical voltage – a process called collision induced dissociation (CID). CID fragments the precursor ion into specific and predictable product ions. The second quadrupole mass filter is set to pass only the specific product ions designated by the user. The most intense ion, the quantifier ion, is used for quantification. The qualifier ion, when found in the correct abundance ratio with the quantifier, is used for confirmation.

Even if an interfering ion is inadvertently allowed to pass through the first quadrupole into the collision cell, the likelihood that the interfering ion would yield the same product ions as the analyte precursor ion is extremely low. In this manner, chemical noise is entirely separated from signal, increasing the signal-to-noise ratio and thus sensitivity.

Compared to performing selected ion monitoring (SIM) using a single quadrupole mass spectrometer, the MRM technique made possible by GC/MS/MS systems offers significantly improved selectivity and sensitivity for the detection of trace-level synthetic cannabinoids in complex matrices such as herbal incense blends.

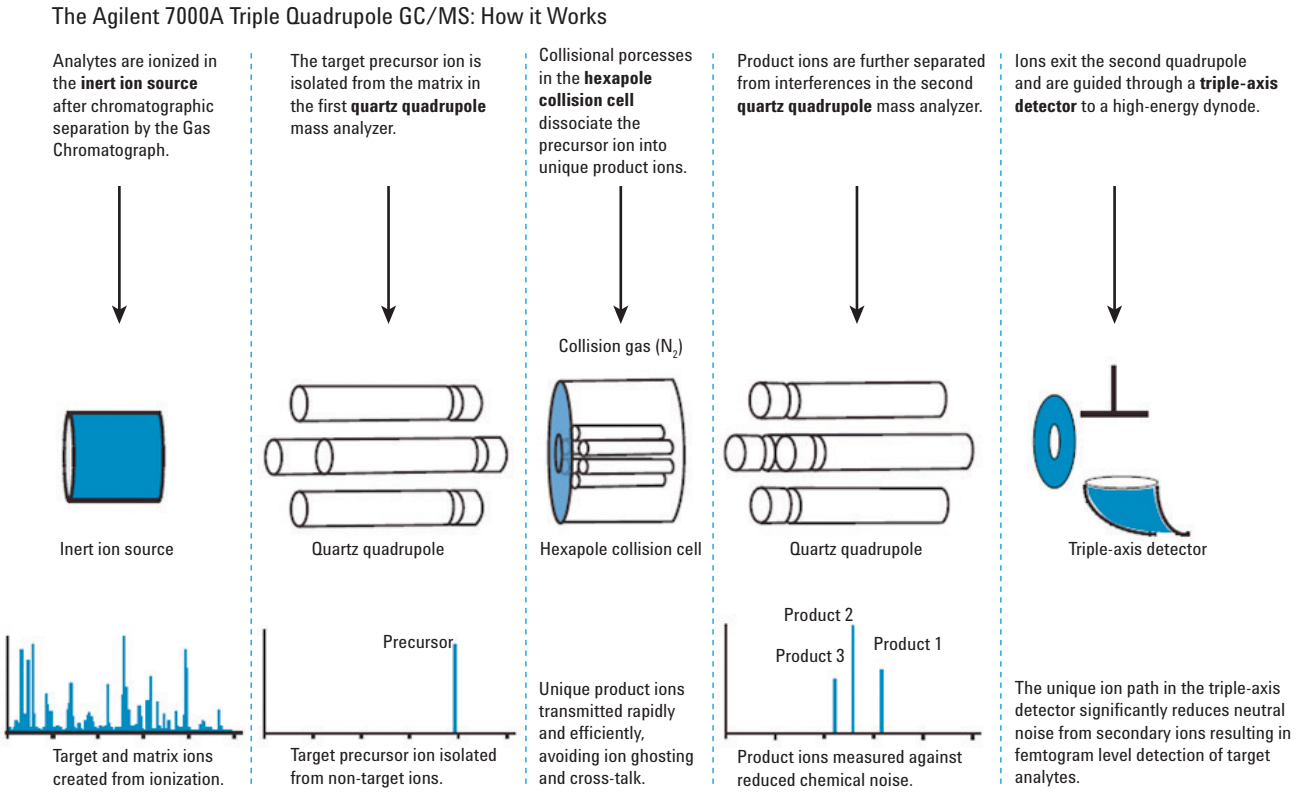


Figure 3. Multiple reaction monitoring (MRM) technique.

The MRM total ion chromatogram (TIC) for 100 ng/mL of the standard mixture is shown in Figure 4. All 17 of the synthetic cannabinoids chosen for analysis were found. Due to the high selectivity of the GC/MS/MS technique, chemical noise is negligible resulting in a very clean TIC.

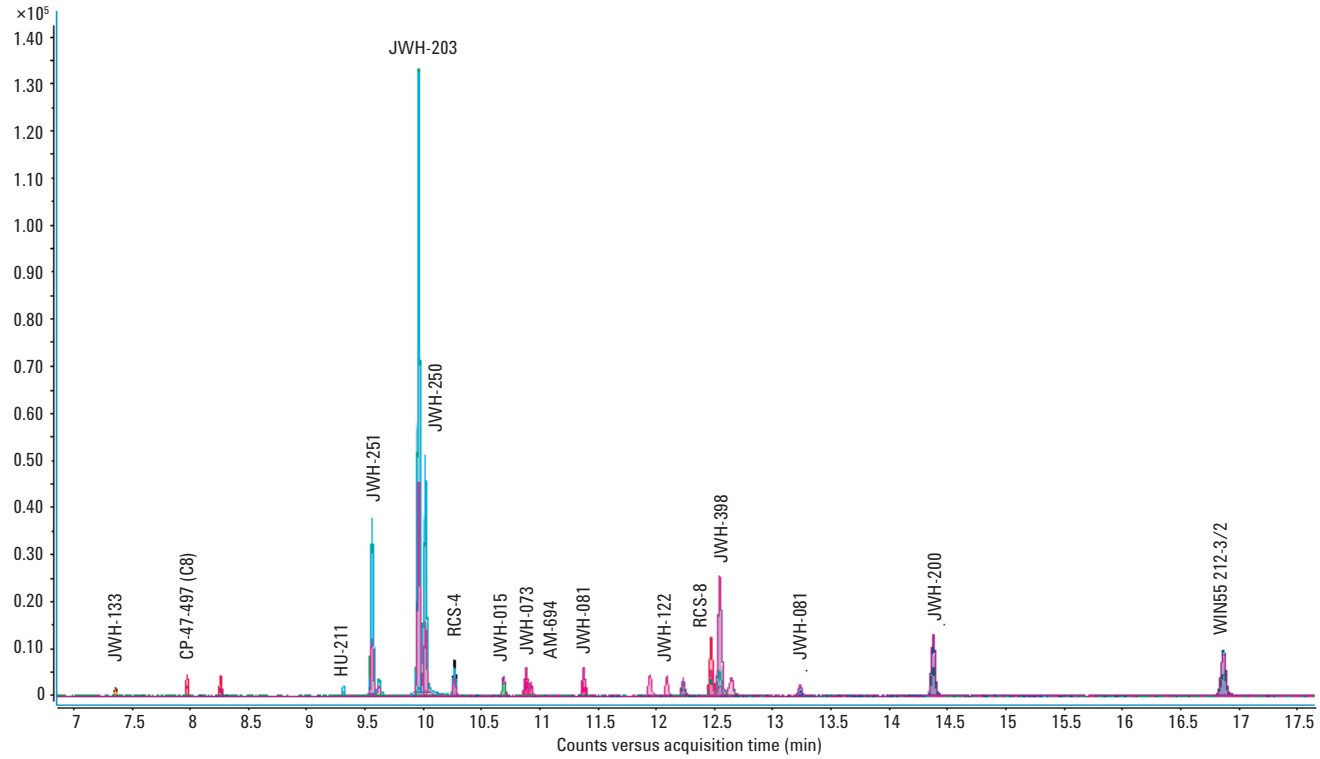


Figure 4. MRM total ion chromatogram for 100 ng/mL of the standard mixture. All 17 synthetic cannabinoid standards were easily found.

Calibration curves were then constructed over the range of 100 – 400 ppb by spiking blank extracted matrix with known reference standards. Replicate injections (n = 3) were made at 100 ppb, 200 ppb, and 400 ppb. The calibration curves for all analytes yielded an average correlation coefficient of linearity (r^2) of 0.99 with standard deviations of 0.012. The average RSD was 13%, 7%, and 6% at 100 ppb, 200 ppb, and 400 ppb, respectively. Levels of quantification as determined by a signal to noise ratio ≥ 10, were determined to range from 1 – 100 ppb in the heavy botanical matrix.

Figure 5 shows the calibration curves for two synthetic cannabinoids with very high activity, JWH-018 and JWH-073 at 100 ng/mL – 400 ng/mL. Typical chromatographic results, for example for JWH-018 at 100 ng/mL, are shown in Figure 6.

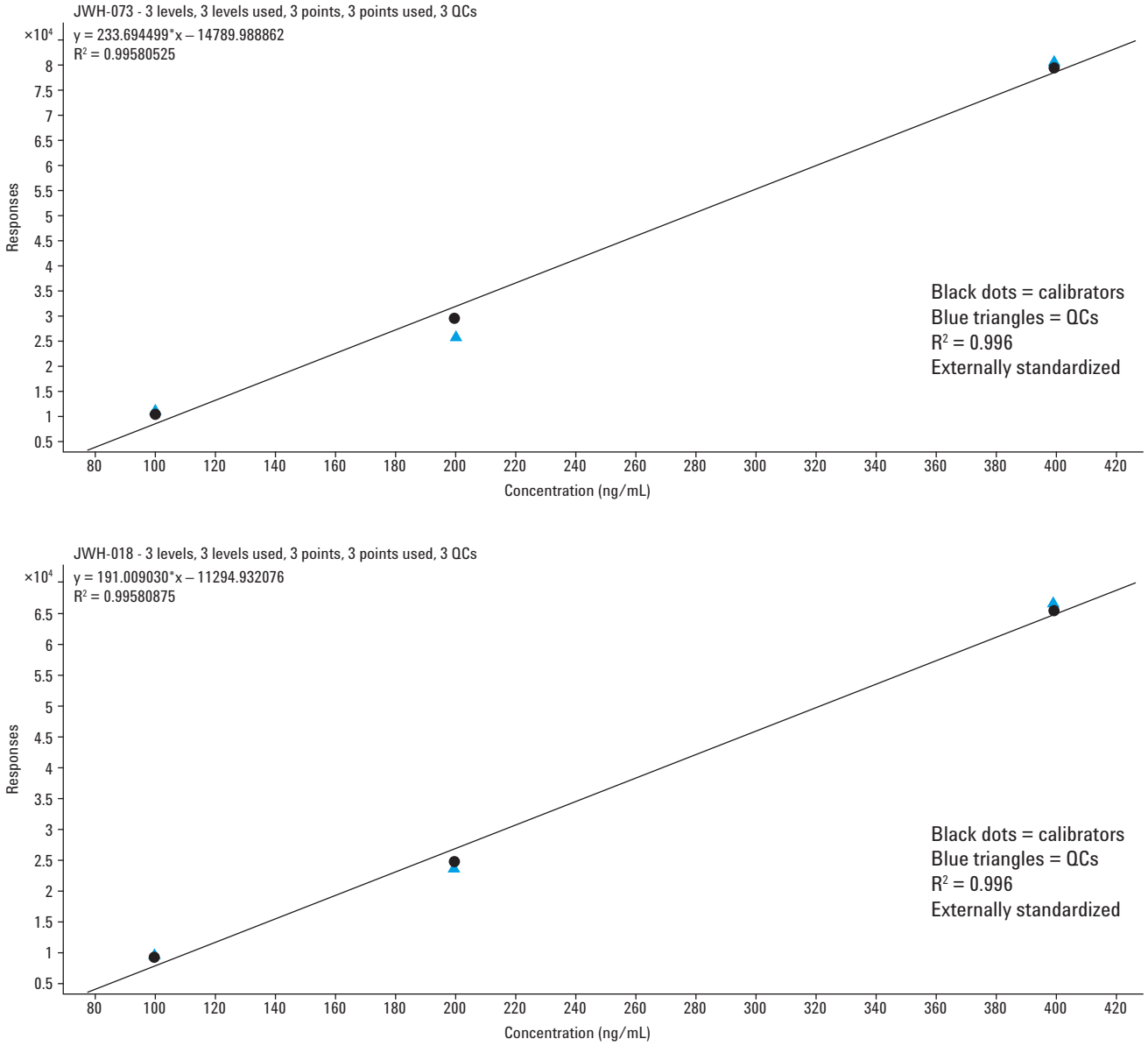


Figure 5. Calibration curves for JWH-018 and JWH-073 show the excellent linearity of the method.

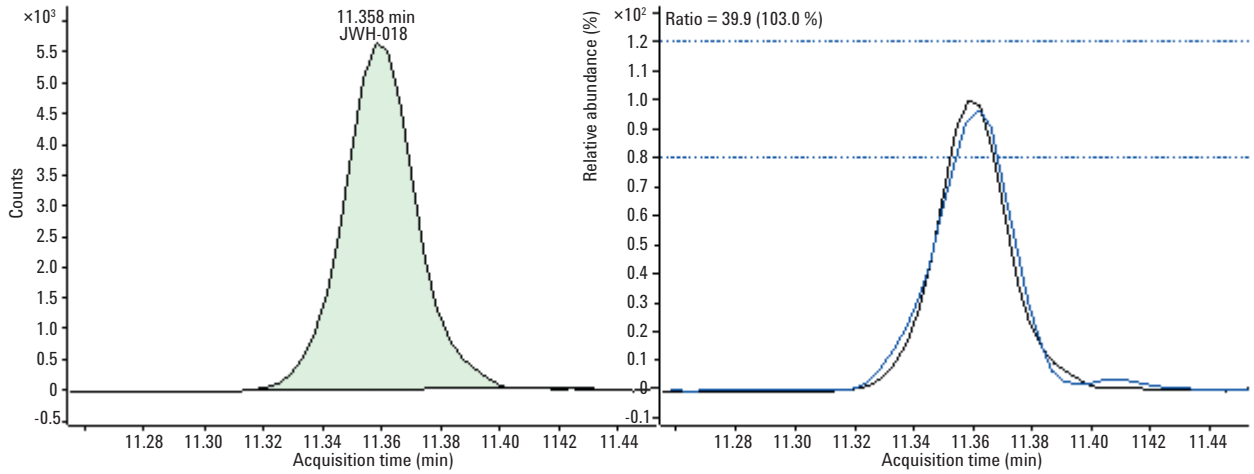


Figure 6. Results for JWH-018 at 100 ng/mL. The shaded peak shows the quantifier ion transition (324 to 254 m/z). The trace shows the qualifier ion transition (341 to 167 m/z) is within the criteria (horizontal lines) established for the method.

Demonstrating the wide variability of herbal blend formulations, JWH-073 and JWH-018 were detected in all of the blends 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 based on area counts alone. All of the blends contained two or more synthetic cannabinoids as confirmed by correct ratio of the qualifying ion to that of the quantifying ion, and the expected retention time.

Conclusion

For the analysis of synthetic cannabinoids in herbal blends, the utility of triple quadrupole MS cannot be overstated. Its ability to negate matrix effects and improve signal-to-noise markedly increase confidence in analytical results. Compared to single quadrupole MS, triple quadrupole MS reduces false negatives and positives, and lowers detection limits, without need for additional post data acquisition processing such as mass spectral deconvolution and review, thereby providing a substantial time savings.

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