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Article

Detection of JWH-018 and JWH-073 by UPLC–MS-MS in Postmortem Whole Blood Casework

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Synthetic cannabinoids have been detected in various herbal blends sold legally in convenience stores, smoke shops, and on the Internet. Many of these compounds have extreme forensic significance. We developed and validated a rapid ultra-performance liquid chromatography–tandem mass spectrometry method for the determination of trace concentrations of two of these compounds, JWH-018 and JWH-073, in human blood. Samples underwent liquid–liquid extraction at pH 10.2 into ethyl ether. Tandem mass spectrometry was performed in positive electrospray ionization mode with multiple reaction monitoring using two transitions and one calculated ion transition ratio for each analyte. Deuterated analogs were used as internal standards. Total run time was 2.6 min. The linear dynamic range was 0.05–50 ng/mL with a limit of detection of 0.01 ng/mL for each analyte. Intra-run imprecision (at two different concentration levels, 2 and 8 ng/mL) was 3.9–10.3% for JWH-018 and 3.5–6.2% for JWH-073. Inter-run imprecision was 6.5–7.2% for JWH-018 and 4.8–5.5% for JWH-073. Intra-run accuracy was 95.9–112.7% for JWH-018 and 92.6–104.7% for JWH-073. Inter-run accuracy was 99.1–107.0% for JWH-018 and 97.7–102.0% for JWH-073. Carryover, exogenous drug interferences, ion suppression and matrix selectivity were also assessed. The method has been applied to postmortem forensic casework received by the laboratory and has proven to be robust and reliable. Concentrations of authentic samples have ranged from 0.1–199 ng/mL for JWH-018 and 0.1–68.3 ng/mL for JWH-073.

Introduction

Many of the synthetic cannabinoids were synthesized by Dr. John W. Huffman's research laboratory at Clemson University in the 1980s and 1990s. Dr. Huffman's research was targeted towards the creation of compounds that were either structural analogs of Δ^9-tetrahydrocannabinol, also known as THC, or compounds that acted on the same receptors in the human body as THC, ultimately for the use in treatment of multiple sclerosis, acquired immunodeficiency syndrome (AIDS), and chemotherapy. Over the course of approximately 20 years, Dr. Huffman's research group synthesized and tested approximately 450 of these synthetic compounds in various groupings (1–4), many of which are considered aminoalkylindoles or naphthoylindoles (5), a term that refers to the chemical structure and the pairing of an indole structural group with an attached alkyl chain to a naphthyl structural group. Each drug in this class shares this overall structure with some modification. The two most well-known are JWH-018 and JWH-073. The chemical structures of these two drugs are listed in Figure 1. The only difference between JWH-018 and JWH-073 is that JWH-018 contains a pentyl alkyl chain, whereas JWH-073 contains a butyl alkyl chain. When compared to THC, a partial receptor agonist that has binding affinity (K_i) values of 40.7 ± 1.7 nm at CB_1 and 36.4 ± 10 nm at CB_2, JWH-018 has a K_i of 9 ± 5 nm at CB_1 and 2.94 ± 2.65 nm at CB_2. JWH-073 has K_i values of 8.9 ± 1.8 nm at CB_1 and 38.0 ± 24.0 nm at CB_2 (5). This stronger binding affinity at both receptors for JWH-018 and the CB_1 receptor for JWH-073 seems to lead to more severe effects than with or after marijuana use, because there have been reports of significant side effects such as tachycardia, increased blood pressure, severe agitation and possible psychosis with hallucinations and delusions due to the drugs (6–9).

In December 2008 and January 2009, study results from a group of scientists in Germany and a group in Austria were released in which they analyzed herbal incense mixtures that were legally being sold to the public, such as Spice silver, Spice gold, Spice diamond, and Yucatan Fire. The chemical analysis of the plant material resulted in the detection of JWH-018 (4). Since 2008, there have been a multitude of reports of detection of synthetic cannabinoids in herbal incense smoking blends. JWH-018 or JWH-073 has been reported in herbal blends in Japan (10, 11), Germany (7, 12, 13), the United Kingdom (14) and the United States (15). None of the manufacturers of the herbal blends disclosed the presence of a drug. Because of the reported side effects and the emergence in legal herbal blends, the United States Drug Enforcement Agency exercised its emergency scheduling powers on March 1, 2011, and temporarily placed JWH-018 and JWH-073, along with three other chemicals, in Schedule I of the Controlled Substances Act (16).

To date, only three analytical methodologies have been published in peer-reviewed literature for the detection of JWH-018 and JWH-073 in serum or blood. The first published method attempted to detect JWH-018 in the serum of a living human by liquid chromatography with tandem mass spectrometry (17). The extraction method was a liquid–liquid extraction with a hexane–ethyl acetate mixture and the overall run time for one specimen 6.5 min. The limit of detection was estimated at 0.07 ng/mL and the limit of quantitation was estimated at 0.21 ng/mL, but both numbers were not truly determined by actual laboratory testing. A second published analytical method included JWH-018, JWH-073 and other compounds, such as JWH-019, JWH-020 and JWH-200 (18). The method employed a liquid–liquid extraction and was validated for the serum matrix, much like the first method, but it increased the reported analyte range and had an 8 min run time. Serum is not a typical matrix that is encountered in a postmortem toxicology laboratory, because it is very improbable (or impossible) to separate hemolyzed blood in to serum. Whole blood is the most common matrix analyzed in our laboratory. Kacinko *et al.* developed and validated an analytical method for the detection

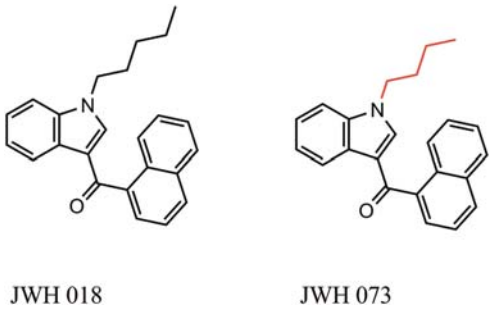

Figure 1. Chemical structures for JWH-018 and JWH-073.

of JWH-018, JWH-073 and two additional compounds in whole blood. This method employed a liquid–liquid extraction with a hexane–ethyl acetate mixture. Total run time was 3.85 min (19). To the authors' knowledge, there have been no other previously published peer-reviewed works describing analytical methods for the detection of these two drugs in whole blood and discussion of concentrations detected in postmortem forensic casework. Due to this scarcity of analytical methodology for whole blood casework and input from our forensic clientele, original research was undertaken in our laboratory to develop methods of detection of JWH-018 and JWH-073 in whole blood.

Experimental

Chemicals, reagents and standards

JWH-018 (100 μg/mL) and JWH-073 (100 μg/mL) reference standards were obtained from Cerilliant Corporation (Round Rock, TX). JWH-018-d9 (1 mg/mL) and JWH-073-d7 (1 mg/mL) reference standards, as well as a second source of JWH-018 (1 mg/mL) and JWH-073 (1 mg/mL) standards, were purchased from Cayman Chemical Company (Ann Arbor, MI). Acetone (ACS grade), acetonitrile (HPLC grade), isopropyl alcohol (ACS grade), methanol (HPLC grade), sodium carbonate buffer salt, sodium bicarbonate buffer salt and concentrated sodium hydroxide were obtained from Fisher Scientific (Pittsburgh, PA). Water used for mobile phase and wash solvents was obtained from a Barnstead Nanopure Diamond Analytical Laboratory Water System (18.2MΩ/cm). Concentrated formic acid (98%) and ethyl ether (ACS grade) were purchased from Sigma-Aldrich (St. Louis, MO). Negative human whole blood was acquired from Utak Laboratories (Valencia, CA) and from past postmortem casework. The gas used with the ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS-MS) system was high-purity nitrogen and argon. Specimen vials and caps were purchased from Micro-Liter Analytical Supplies (Suwanee, GA). JWH-018 and JWH-073 reference standards were diluted to a concentration of 1 μg/mL with methanol. The calibration curve and quality control specimens were prepared by spiking known amounts of standard into negative human blood, which had previously screened negative for drugs of abuse, therapeutic drugs, and other chemicals by a combination of enzyme-linked immunosorbent assay () and UPLC with time of flight mass spectrometry (ToF) for other drugs of abuse and therapeutic drugs. The internal standard solution was prepared by adding 25 μL of each of the 1 mg/mL deuterated analog reference standards to 1 L of acetonitrile. The sodium carbonate buffer, pH 10.2, was prepared by adding 7.47 g of sodium bicarbonate buffer salt and 1.17 g of sodium carbonate buffer salt to 1 L of deionized (DI) water and adjusting the pH to 10.2 with 1M sodium hydroxide. Mobile phase A (0.1% formic acid in DI water) was prepared by adding 2 mL of concentrated formic acid to 2 L of DI water. Mobile phase B (0.1% formic acid in acetonitrile) was prepared by adding 2 mL of concentrated formic acid to 2 L of acetonitrile. Weak needle wash was prepared by adding 100 mL of acetonitrile and 10 mL of concentrated formic acid to 1.9 L of DI water. Strong needle wash was prepared by adding 900 mL of isopropyl alcohol and 200 mL of acetone to 900 mL of acetonitrile.

Specimen collection

Blood specimens were collected during autopsy in 60 mL polypropylene bottles or 10-mL polypropylene tubes containing sodium fluoride and EDTA as a preservative and sent to the laboratory at ambient temperature for routine toxicological analysis and JWH-018/JWH-073 analyses. Routine toxicological analyses for blood included an immunoassay (ELISA) screen for classical cannabinoids and opiates/opioids and a UPLC–ToF screen for 300 other abused drugs, therapeutic drugs and metabolites. Specimens were either analyzed immediately after receipt by the laboratory or stored in refrigerated conditions (4°C) until analysis. Some older casework that had been held in frozen (−10°C) conditions was thawed and analyzed.

Organic extraction procedure

Human whole blood was extracted by the following liquid–liquid extraction procedure. A 500-μL aliquot of human blood specimen was added to a tube, along with 50 μL of the internal standard working solution and 1 mL of sodium carbonate buffer, pH 10.2. The mixture was vortexed for 10 s. Five milliliters of ethyl ether was added and the samples were gently mixed for 5 min and centrifuged for 10 min at 5°C and 3,750 RPM. The organic layer was immediately transferred a new tube and immediately evaporated to complete dryness under nitrogen gas flow. The residue was reconstituted in 200 μL of acetonitrile–DI water (50:50), vortexed and transferred to a plastic autosampler vial.

Instrumental analysis

Chromatography

The separation module was a Waters (Milford, MA) Acquity UltraPerformance Liquid Chromatograph. The system consisted of a sample manager, solvent manager, and column manager. UPLC separation was performed by injecting ten microliters of specimen on a Waters Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.8 μm particle size) at a temperature of 50°C. Chromatographic separation was achieved by gradient elution at an flow rate of 0.5 mL/min. Mobile phases used during analysis were 0.1% formic acid in DI water (A) and 0.1% formic acid in acetonitrile (B). A summary of the inlet method is

Table I
The Elution Gradient

Total time (min)	Flow rate (mL/min)	% A	% B
Initial	0.500	48	52
0.10	0.500	48	52
1.90	0.500	0	100
2.30	0.500	0	100
2.31	0.500	48	52
Retention times (min)	JWH-018 (1.45) JWH-073 (1.35) JWH-018 d9 (1.45), JWH-073 d7 (1.35)		

Table II
The Mass Spectrometer Method

Analyte	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)	Type
JWH-018	342.2	155.0	50	26	Quantifying
	342.2	126.9	50	46	Qualifying
JWH-073	328.1	155.0	45	24	Quantifying
	328.1	127.0	45	46	Qualifying
JWH-018 d9	351.2	154.9	45	26	Internal standard
JWH-073 d7	335.2	127.0	40	48	Internal standard

shown in Table I. Total run time for one specimen was 2.6 min. After each injection, the system was washed with 1 mL of strong needle wash and 1 mL of weak needle wash. Retention time of JWH-018 was 1.45 min, while retention time of JWH-073 was 1.35 min.

Mass spectrometry

Electrospray ionization mass spectrometry (ESI-MS) was performed on a Waters tandem quadrupole detector (TQD) in positive ionization multiple reaction monitoring mode (MRM). Capillary voltage was 0.6 kV. Cone voltage was 41 V. Extractor voltage was 3 V. Source temperature was 150°C and the desolvation temperature was 450°C. The desolvation gas (nitrogen) and cone gas (nitrogen) were set to 900 and 50 L/min, respectively. Collision gas (argon) flow was 0.30 mL/min. Collision cell pressure was 8.5×10^{-3} mbar of argon. Two MRM transitions were monitored for the analytes of interest and one MRM transition was monitored for each of the internal standards. Dwell times were 0.02 s for all transitions. Average ion ratio for the JWH-018 transitions was 1.1. The average ion ratio for the JWH-073 transitions was 1.1. The mass spectrometer method is summarized in Table II.

Analytical method validation protocol

The analytical method for determination of JWH-018 and JWH-073 in blood specimens was validated as a quantitative assay. Linearity, including limit of detection (LOD), lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ), imprecision and accuracy, matrix selectivity, exogenous drug interferences, ion suppression and carryover were assessed.

Linearity was assessed by spiking six replicates of negative human blood with JWH-018 and JWH-073 at concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 and 50 ng/mL. The first replicate of each concentration was set as a standard forming a calibration curve; a linear regression was applied with a $1/x$ weighting factor. The other five replicates at each level were quantitated using the calibration curve. Acceptance criteria are that all concentrations points comprising the linearity should have a coefficient of variation (CV) less than 15.0%, except the LLOQ, which should have a CV less than 20.0%. The percent accuracy for each concentration level tested should be within 85.0–115.0%, except the LLOQ, which should be within 80.0–120.0%. The R^2 value should be greater than or equal to 0.990. The LLOQ is defined as the lowest calibration curve point that has acceptable ion transition ratios ($\pm 20\%$), acceptable retention time (± 0.05 min from the deuterated analog's retention time), signal to noise ratio of ten or greater, and passes data acceptance criteria for imprecision and accuracy. The limit of detection is defined as the lowest calibration curve point tested that had acceptable ion transition ratios ($\pm 20\%$), acceptable retention time (± 0.05 min from the deuterated analog's retention time), and a signal to noise ratio of at least three or greater.

Imprecision and accuracy were assessed by extracting a batch of specimens that included a calibration curve (0.05–50 ng/mL), a negative quality control specimen, five low quality control specimens (2 ng/mL) and five high quality control specimens (8 ng/mL) over the course of five days. Statistical analysis was completed to determine intra-run accuracy, inter-run accuracy, intra-run imprecision and inter-run imprecision at the two quality control specimen concentrations. Acceptance criteria are the average intra-run and inter-run imprecision should be less than 15.0% CV for all quality control specimens and the average intra-run and inter-run percent accuracy should be within 80.0–120.0% for both quality control specimens. Each calibration curve's R^2 value should be greater than or equal to 0.990.

The presence of endogenous compounds, such as metabolites and matrix components in the biological matrix, may affect the ability of the analytical method to measure the analytes of interest with accuracy and precision. Matrix selectivity was assessed by spiking ten sources of negative human blood and one source of DI water with JWH-018 and JWH-073 pre-extraction at concentrations of 0 (unspiked), 0.2, 5 and 8 ng/mL. The extraction was completed as normal. The sample responses of the spiked sources of matrix were compared to the sample responses of the spiked DI water source at each concentration level and a CV was calculated. A percent accuracy was determined by dividing the response of the analyte in the matrix spike by the response of the analyte in the DI water spike and converting to percent. Acceptance criteria are the CV of the sample responses should be less than or equal to 15.0% at each concentration analyzed and the percent accuracy for each analyte in comparison to the DI water spike should be between 80.0–120.0% at each concentration. Also, 80.0% of the spiked samples should have measured results within $\pm 20.0\%$ of the DI water spikes for that concentration level.

Analytical methodology must be free from exogenous interferences from drugs that might be present in the specimen, such as illicit drugs, over-the-counter drugs or other prescribed drugs. Any interfering analyte might lead to the possibility of a false positive result. Interferences from other drugs and metabolites were assessed by spiking human blood pre-extraction

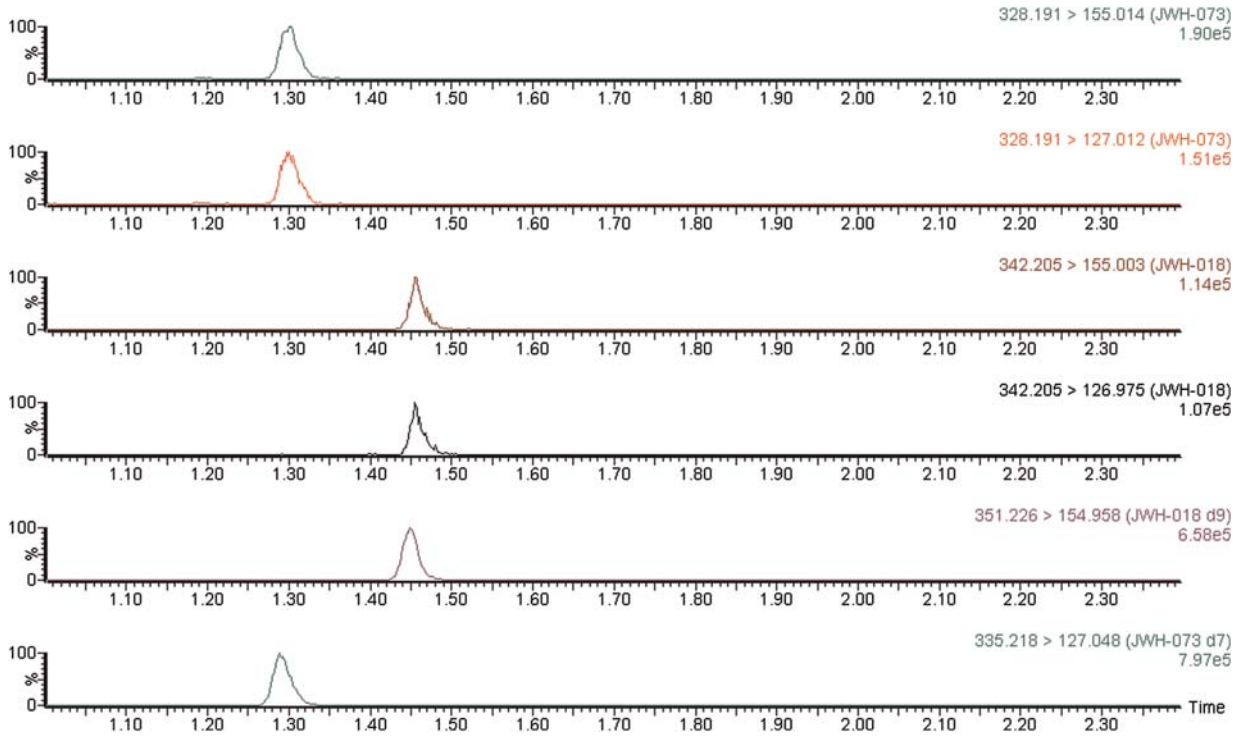

Figure 2. Analytes of interest in whole blood at the limit of quantitation (0.05 ng/mL) along with internal standards.

with various drugs at 1 μg/mL. The interference specimens were extracted as normal and analyzed for the presence of interfering analyte signals at the correct retention time for JWH-018 and JWH-073. If a quality control specimen was determined to have an interfering analyte, then the analytes in the specific specimen would be spiked individually in to human blood and the interference would be specifically identified and documented. Acceptance criteria are that no interfering signal be present for the ion transitions of JWH-018 and JWH-073.

Interfering substances present in the specimen may lead to a competition for charge from the mass spectrometer and analyte signal suppression. Using a deuterated analog of the analyte of interest may help alleviate ion suppression effects. Ion suppression was assessed by spiking ten sources of negative human blood and one source of DI water with JWH-018 and JWH-073 at 0 (unspiked), 0.2, 5 and 8 ng/mL post-extraction, along with 5 ng/mL JWH-018-d9 and JWH-073-d7. Matrix effect (ME) was calculated as the quotient of the area of the analyte in the sample to the area of the analyte in the spiked water blank. The response effect (RE) was calculated as the quotient of the mean sample responses to the spiked water blank response. The response from the spiked water blank was not included in the average response. Acceptance criteria are that the ME for each analyte and internal standard at each concentration level should have a CV less than or equal to 30.0%. The CV of the response effects for each analyte at each concentration should be less than or equal to 10.0%. Also, 80.0% of the samples should show a response effect between 0.8–1.2.

Carryover was assessed by spiking JWH-018 and JWH-073 in blood specimens pre-extraction at 1,000 ng/mL and injecting the spiked specimen, followed by five consecutive extracted blank matrix extracts. If carryover was determined to occur, then the concentration would be decreased by half and the experiment repeated. Acceptance criterion is no presence of a quantifiable analyte signal in the extracted blank matrix extracts.

Validation results and application of the method

The blood assay was linear from 0.05 ng/mL (LLOQ) to 50 ng/mL (ULOQ), with an LOD of 0.01 ng/mL for both JWH-018 and JWH-073. Figure 2 shows the chromatography of all transitions at the LLOQ. The LOD was the lowest value tested in the study; no estimation of LOD was undertaken. All R^2 values were 0.996 or greater. For JWH-018, the intra-run CV values were between 3.9–10.3% and the inter-run % CV values were between 6.5–7.2%. The accuracy values were between 95.9–112.7%. For JWH-073, the intra-run % CV values were between 3.5–6.2% and the inter-run % CV values were between 4.8–5.5%. The accuracy values were between 92.6–104.7%. Although some matrix effects were observed, the deuterated analogs of JWH-018 and JWH-073 compensate for any suppression of signal. The internal standard tracks with the analyte in response. Of the 168 drugs and metabolites tested, no exogenous drug interferences were noted during the validation. Isobaric compounds (JWH-015/JWH-073) are chromatographically separated (JWH-015's retention time is 1.25 min). The resolution between these two compounds is shown in Figure 3. No carryover was detected in the blank extracts injected immediately following a specimen spiked with 1,000 ng/mL of JWH-018 and JWH-073. Figure 4 details the chromatography of the first blank extract injected after the 1,000 ng/mL specimen. Blood assay validation statistics are summarized in Tables III–V.

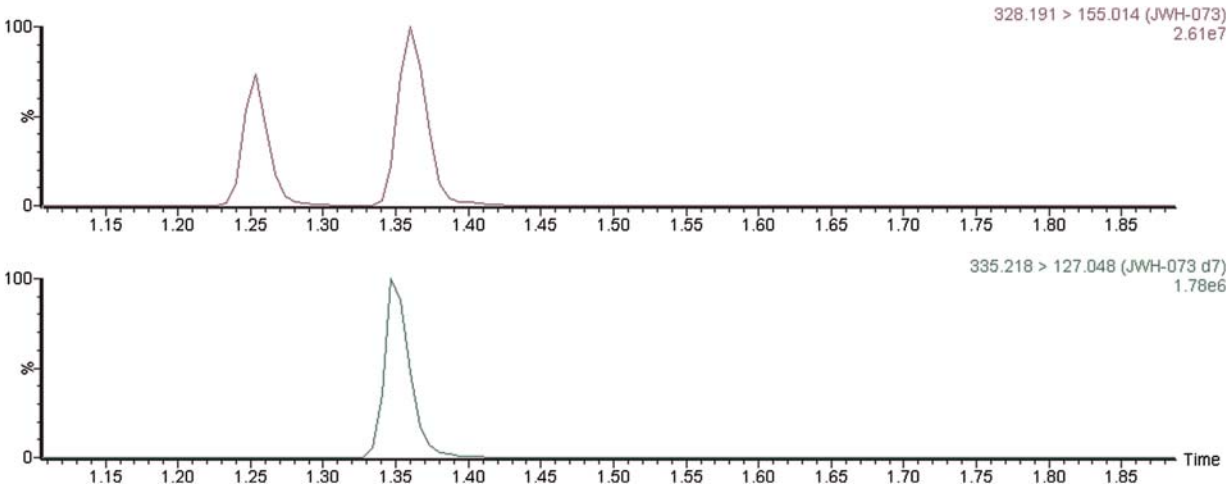

Figure 3. Chromatographic resolution between isobaric compounds JWH-015 and JWH-073.

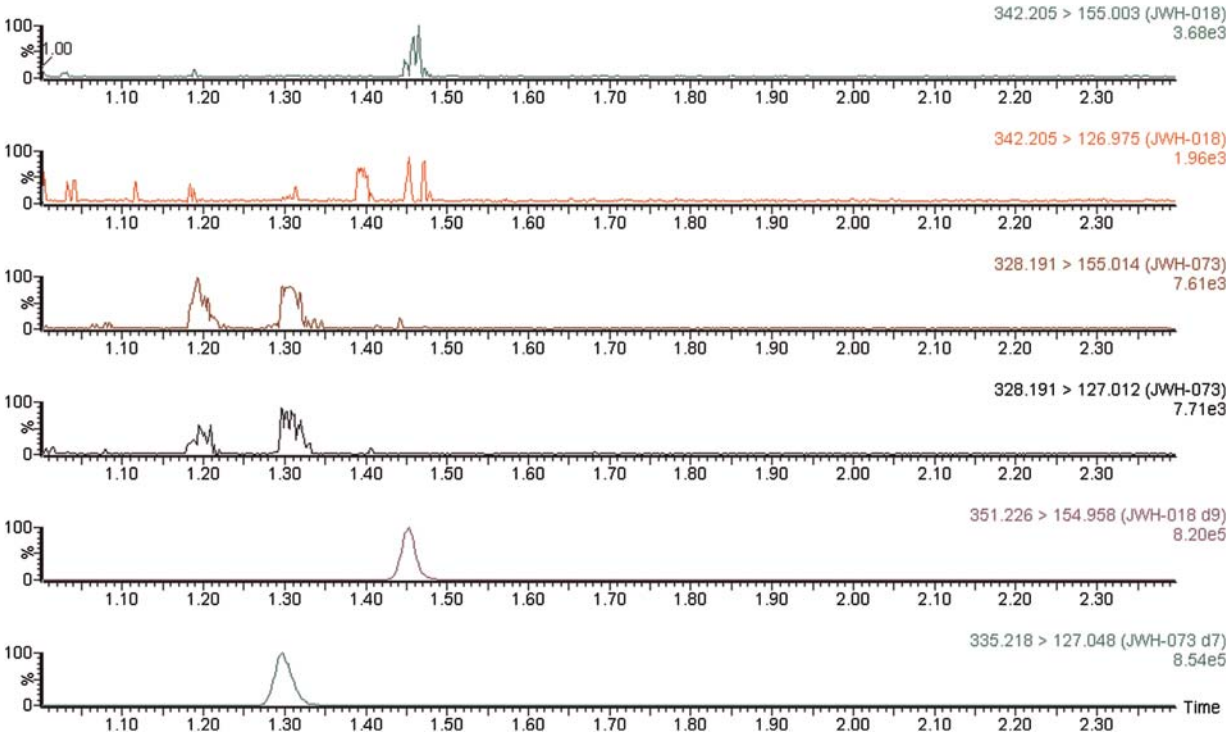

Figure 4. First blank specimen injected after a whole blood specimen spiked at 1,000 ng/mL.

Discussion

At present, 45 postmortem blood cases have been analyzed for the presence of JWH-018 and JWH-073. We have detected one or both of the parent drugs in 18 of the 45 forensic cases, which constitutes a 40.0% positivity rate. JWH-018 was detected in all 18 of the positive cases, with none containing only JWH-073. Results of the cases are summarized in Table VI. The concentrations vary widely. Any sample that yielded a concentration greater than the ULOQ was diluted and rerun to achieve a valid result within the linear range. Results for JWH-018 range from 0.1–199 ng/mL (mean, 17.5 ng/mL), while results for JWH-073 range from 0.1–68.3 ng/mL (mean, 8.7 ng/mL). Three of the highest concentration JWH-018 and/or JWH-073 cases are described in detail.

Case One

A 57-year-old male (325 lbs) was found unresponsive by emergency medical services at a business that he owned after an employee's girlfriend called on the phone and determined that "he did not sound right". Typical resuscitation efforts were undertaken, including administration of naloxone (Narcan).

Table III
Imprecision, Accuracy and Linearity

Analyte	Intra-run accuracy	Inter-run accuracy	Intra-run imprecision	Inter-run imprecision	Linearity (ng/mL)
JWH-018					0.05–50
2 ng/mL	103.3–112.7%	107.0%	5.1–7.9%	7.2%	LOD (0.01)
8 ng/mL	95.9–101.8%	99.1%	3.9–10.3%	6.5%	
JWH-073					0.05–50
2 ng/mL	99.1–104.7%	102.0%	4.0–5.3%	4.8%	LOD (0.01)
8 ng/mL	92.6–100.3%	97.7%	3.5–6.2%	5.5%	
$N = 25$					

Table IV
Matrix Selectivity and Ion Suppression

Analyte	Matrix Effect (Mean)	Matrix effect (% CV)	Response effect	Response effect (% CV)
JWH-018				
0.2 ng/mL	0.90	7.4	0.91	5.5
5 ng/mL	0.94	7.1	0.96	2.6
8 ng/mL	0.98	5.6	0.95	2.6
JWH-073				
0.2 ng/mL	1.05	4.7	1.05	4.5
5 ng/mL	0.98	3.6	0.95	3.1
8 ng/mL	1.03	3.0	0.96	1.5

Table V
Exogenous Drug Interferences Tested

QC	Concentration (ng/mL)	Analytes
1	500	AM694, AM2201, cannabidiol, cannabinol, CP47,497, CP47,497-C8, HU-210, JWH-015, JWH-019, JWH-081, JWH-098, JWH-122, JWH-200, JWH-201, JWH-203, JWH-210, JWH-250, JWH-251, JWH-398, RCS-4, RCS-8, THC, THC-COOH
2	1,000	10-Monohydroxyoxcarbazepine, carbamazepine, carbamazepine epoxide, felbamate, gabapentin, lamotrigine, levetiracetam, mephobarbital, phenytoin, pregabalin, primidone, topiramate, valproic acid, zonisamide
3	1,000	Amitriptyline, bupropion, citalopram, clomipramine, desipramine, doxepin, duloxetine, fluoxetine, imipramine, mCPP, mirtazapine, norclomipramine, nordoxepin, norfluoxetine, norsertraline, nortriptyline, norvenlafaxine, paroxetine, sertraline, trazodone, trimipramine, venlafaxine,
4	1,000	9-Hydroxyrisperidone, aripiprazole, chlorpromazine, clozapine, dehydroaripiprazole, haloperidol, norclozapine, olanzapine, quetiapine, risperidone, ziprasidone
5	1,000	Acetaminophen, baclofen, cyclobenzaprine, etodolac, fenoprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, meloxicam, metaxalone, methocarbamol, naproxen, oxaprozin, piroxicam, salicylic acid, sulindac
6	1,000	Amphetamine, BDB, Butylone, cathinone, ethylone, flephedrone, MDA, MDEA, MDMA, MDPV, mephedrone, methamphetamine, methcathinone, methedrone, methylone, phentermine,
7	1,000	7-Aminoclonazepam, 7-aminoflunitrazepam, alprazolam, chlordiazepoxide, clonazepam, demoxepam, deskalkylflurazepam, diazepam, flumazenil, flunitrazepam, flurazepam, lorazepam, medazepam, midazolam, nordiazepam, oxazepam, phenazepam, temazepam, triazolam
8	1,000	6-MAM, buprenorphine, codeine, diacetylmorphine, dihydrocodeine, EDDP, fentanyl, hydrocodone, hydromorphone, methadone, morphine, naloxone, norbuprenorphine, noscapine, oxycodone, oxymorphone, tapentadol
9	1,000	Benzoylecgonine, bupivacaine, carisoprodol, cocaethylene, cocaine, etomidate, ketamine, lidocaine, meprobamate, norpropoxyphene, notramadol, propoxyphene, rocuronium, tramadol
10	1,000	Amobarbital, barbital, butabarbital, butalbital, chlorpheniramine, dextromethorphan, diphenhydramine, doxylamine, hexobarbital, methohexital, PCP, pentobarbital, phenobarbital, secobarbital, thiopental

Table VI
Postmortem Results for JWH-018 and JWH-073 in 18 Forensic Cases

Case number	Blood source	JWH-018 (ng/mL)	JWH-073 (ng/mL)
1	Cardiac	199	None detected
2	Cardiac	19.6	68.3
3	Cardiac	83.3	None detected
4	IVC	0.2	0.2
5	Femoral	0.1	None detected
6	Cardiac	0.8	0.3
7	Femoral	1.0	0.3
8	Cardiac	2.2	None detected
9	Femoral	1.2	0.4
10	IVC	0.5	0.3
11	Subclavical	0.6	None detected
12	Subclavical	0.1	0.1
13	IVC	0.4	0.2
14	Cardiac	0.3	None detected
15	Cardiac	0.1	None detected
16	Cardiac	0.2	None detected
17	Subclavical	5.5	None detected
18	Cardiac	0.5	None detected

The man remained in asystole after the attempts to revive him and was pronounced deceased in the emergency room after arrival. Witness accounts describe that the man had smoked herbal incense, Spice, as well as a white powdery substance thought to be JWH-018. The postmortem examination revealed an enlarged heart. Postmortem cardiac blood was collected during autopsy and submitted for toxicological analysis. The cardiac blood concentration of JWH-018 was 199 ng/mL. In addition, clonazepam (5.5 ng/mL), 7-aminoclonazepam (56.6 ng/mL), methadone (887 ng/mL), EDDP (115 ng/mL), morphine (122 ng/mL), pregabalin (1.8 μg/mL), topiramate (4.1 μg/mL) and naloxone (qualitative) were detected in the blood specimen. The deceased held valid prescriptions for clonazepam, methadone, morphine, pregabalin and topiramate

Case Two

A 52-year-old male was found nude and unresponsive on the floor in his residence at his parent's home. A K2 brand herbal blend packet was found at the scene. The decedent was known to be an avid herbal incense user. Postmortem cardiac blood was collected at autopsy and submitted for toxicological analysis. The cardiac blood concentration of JWH-018 was 19.6 ng/mL and JWH-073 was 68.3 ng/mL. No other substances were detected in the blood specimen. Figure 5 details the chromatographic peaks for JWH-018, JWH-073 and the deuterated analogs in this specimen.

Case Three

A 29-year-old male committed suicide by exsanguination following self-administration (smoking) of a K2 herbal blend. Suicidal tendencies had been noted in the deceased's history. Other than the fact that the decedent was an avid user, no other information was given. Postmortem cardiac blood was collected during autopsy and submitted for toxicological analysis. The cardiac blood concentration of JWH-018 was 83.3 ng/mL. No other substances were detected in the blood specimen.

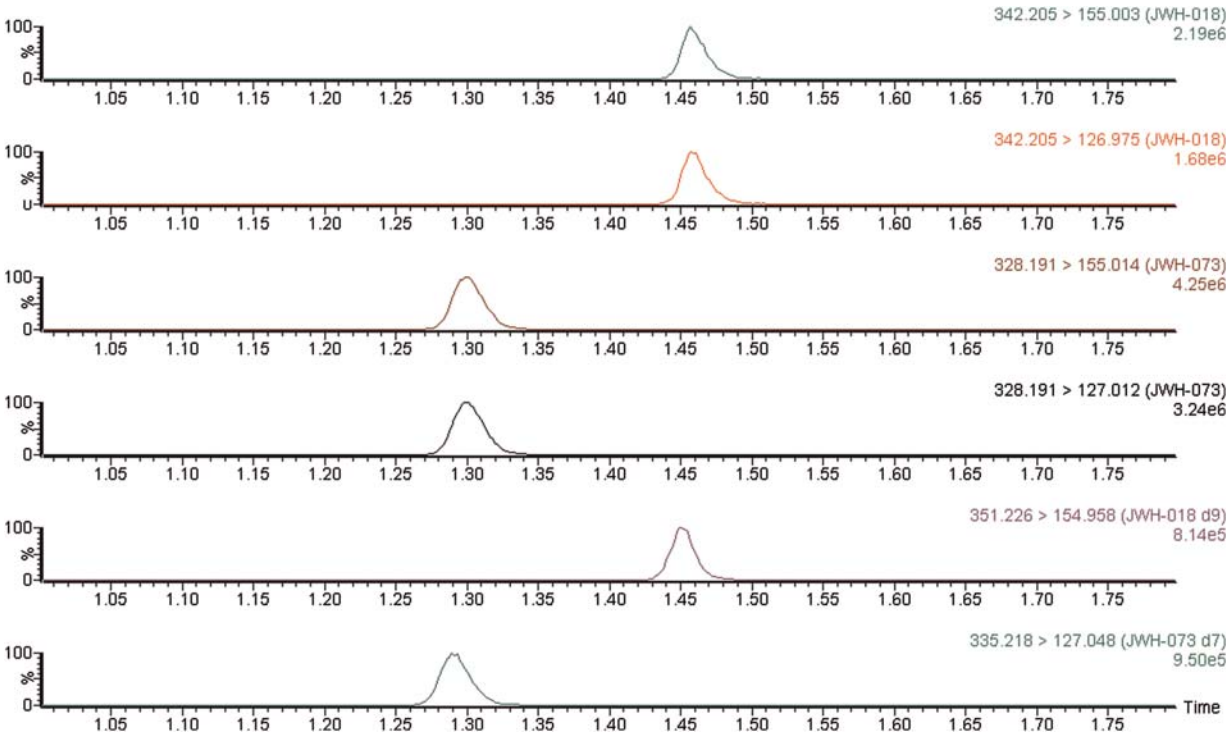

Figure 5. Chromatogram of authentic specimen: Case 2, JWH-018 (19.6 ng/mL), JWH-073 (68.3 ng/mL).

The three cases discussed involve higher concentrations of JWH-018 and JWH-073 than were detected in the other 15 forensic cases. These high concentrations have not been reported in the few studies reported in literature and actually are contradictory to any published data on detected concentrations. The blood specimens tested in the previously described cases were cardiac blood; none of the high concentration specimens were blood from peripheral sites of the body, therefore, potential postmortem redistribution must be considered or even assumed. The controlled study published by Teske *et al.* showed peak serum concentrations of JWH-018 occurring at 5 min after administration, rapidly declining to 0.16 and 0.13 ng/mL at 6 h and not detectable after 24 h (17). Another difference may be that during the controlled administration study, the two subjects smoked herbal incense plant material rolled in a cigarette and were not historical or avid users of the drug. The serum specimens analyzed by Dresen *et al.* had a JWH-018 concentration range of 0.3–8.17 ng/mL and a JWH-073 concentration range of 0.23–0.6 ng/mL (18). The single blood specimen from a controlled dosing study analyzed by Kacinko *et al.* had concentrations of 4.8 ng/mL for JWH-018 and 4.2 ng/mL for JWH-073 at 19 min and concentrations of 0.2 ng/mL for JWH-018 and 0.2 ng/mL for JWH-073 at 199 min post-dosing (19). Case One discussed previously involved the combination of smoking plant material and concentrated powder form of JWH-018, which may have resulted in the extremely elevated concentration of drug detected. Also, much like THC, these drugs are relatively nonpolar and may be lipophilic, which leads one to believe that they could accumulate in fat tissue and be released over time. Obviously, more controlled research and publication of postmortem concentrations is needed in this area.

Conclusion

JWH-018 and JWH-073 are members of the naphthoylindole group of synthetic cannabinoids, which have been detected in various herbal incense smoking blends that can be readily purchased via convenience stores, gas stations and the Internet. The DEA has temporarily placed JWH-018 and JWH-073 into Schedule I of the Controlled Substances Act. These compounds are agonists at the cannabinoid receptors in the body and can produce some severe side effects. For these reasons, these compounds are of forensic significance and the modern forensic toxicology laboratory must be able to detect them in the typical biological matrices, including whole blood. More research is needed in this area, because only a few studies for the detection of these drugs in biological matrices have been published in peer-reviewed scientific journals, and this is the first study to detail postmortem whole blood analysis and concentrations. We developed and validated an accurate, precise and reproducible method of detection of JWH-018 and JWH-073 in human blood by liquid–liquid extraction and UPLC–MS-MS. Further investigation by the laboratory will include the possible addition of other relevant synthetic cannabinoid compounds.

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