Determination of naphthalen-1-yl-(1-pentyllindol-3-yl)methanone (JWH-018) in mouse blood and tissue after inhalation exposure to ‘buzz’ smoke by HPLC/MS/MS

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Abstract

The disposition of the cannabimimetic naphthalen-1-yl-(1-pentyllindol-3-yl)methanone (JWH-018) in mice following inhalation of the smoke of the herbal incense product (HIP) ‘Buzz’ is presented. A high-pressure liquid chromatography with electrospray ionization triple quadrupole mass spectrometer (HPLC/MS/MS) method was validated for the analysis of JWH-018 in the specimens using deuterated Δ⁹-tetrahydrocannabinol (d3-THC) as the internal standard. JWH-018 was isolated by cold acetonitrile liquid–liquid extraction. Chromatographic separation was performed on a Zorbax eclipse XDB-C18 column. The assay was linear from 1 to 1000 ng/mL. Six C57BL6 mice were sacrificed 20 min after exposure to the smoke of 200 mg ‘Buzz’ containing 5.4% JWH-018. Specimen concentrations of JWH-018 were: blood, 54–166 ng/mL (mean 82 ± 42 ng/mL); brain, 316–708 ng/g (mean 510 ± 166 ng/g); and liver, 1370–3220 ng/mL (mean 1990 ± 752 ng/mL). The mean blood to brain ratio for JWH-018 was 6.8 and ranged from 4.2 to 10.9. After exposure, the responses of the mice were consistent with cannabinoid receptor type 1 activity: body temperatures dropped 7.3 ± 1.1 °C, and catalepsy, hyperreflexia, straub tail and ptosis were observed. The brain concentrations and physiological responses are consistent with the hypothesis that the behavioral effects of ‘Buzz’ are attributable to JWH-018.

Keywords

JWH-018; HPLC/MS/MS; Herbal Incense product

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**Introduction**

Over the past decade various herbal incense products (HIPs) have become available via the internet and at various retail outlets throughout Europe, the USA and Japan (European Monitoring Centre for Drugs and Drug Addiction, 2009). HIPs claim to be blends of plant materials and incense and are labeled ‘not for human consumption’; however, they are allegedly capable of producing a marijuana-like high when smoked (European Monitoring Centre for Drugs and Drug Addiction, 2009). HIPs are usually sold in colorful foil packets of 3–5 g of material under numerous attractive or exotic names such as: Spice Gold, Magic Gold, K-2, Summit, Lion’s Tail, Buzz, Pulse, Chill Out and Yucatan Fire. Chemical analyses performed on HIPs have identified a variety of synthesized cannabimimetic compounds present (European Monitoring Centre for Drugs and Drug Addiction, 2009; Auwärter et al., 2009; Hudson et al., 2010; Lindigkeit et al., 2009; Uchiyama et al., 2009a, 2009b, 2010; Zuba et al., 2011). Many of these compounds interact with the cannabinoid receptor type 1 (CB1) receptors in the central nervous system in a similar manner to Δ9-tetrahydrocannabinol (THC), the main active compound in marijuana (Aung et al., 2000; Atwood et al., 2010; Compton et al., 1992; Huffman and Dai, 1994; Wiley et al., 1998). These synthetic compounds were initially developed as pharmacological tools to investigate cannabinoid receptor(s) in the brain and other tissues and as potential therapeutic compounds (Zhang et al., 2006). The most commonly encountered cannabimimetics in HIPs are cyclohexylphenol derivatives such as CP 47,497 (Weissmann et al., 1982) and naphthoylindole compounds JWH-018 and JWH-073 (Huffman et al., 2005). These compounds are a new group of designer drugs, used specifically to circumvent drug laws associated with marijuana use and sale, although they have been temporarily categorized as Schedule 1 drugs by the Drug Enforcement Agency (2010).

JWH-018 appears to be the most popular constituent in HIPs and as such is presently the most studied. JWH-018 has been recently detected in the serum of two subjects after smoking 100 mg (female subject) and 150 mg (male subject) of the HIP ‘Smoke’, which contained 2.9% JWH-018 (Teske et al., 2010). Serum concentrations were 8 and 10 ng/mL within 5 min of smoking, respectively; however, they dropped below the assay limit of quantitation of 0.5 ng/mL within 6 h. Another study of serum specimens from 80 subjects provided by various clinical and forensic sources detected JWH-018 in nine samples at concentrations ranging from 0.30 to 8.1 ng/mL with a mean of 1.84 ng/mL (Sebastian et al., 2011). Grigoryev et al. (2011) studied the metabolism of JWH-018 in humans and mice. They found that the major urinary metabolites of JWH-018 in man were glucuronides of various ring monohydroxylated metabolites, while dealkylated glucuronides of monohydroxylated metabolites predominated in mice. These metabolites of JWH-018 were also identified in the urine of three people arrested for public intoxication (Sobolevsky et al., 2010).

There is little pharmacological or toxicological data concerning the disposition of illicitly used cannabimimetics in laboratory animals or humans. This study was undertaken in order to gain a better understanding of the pharmacokinetic phase of cannabimimetic intoxication and determine the disposition of JWH-018 in blood, brain and liver of mice after controlled exposure to smoke from the HIP ‘Buzz’. JWH-018 was identified and quantified in whole blood and tissues by high-pressure liquid chromatography with electrospray ionization triple quadrupole mass spectrometers (HPLC/MS/MS). At present there is not a procedure that addresses JWH-018 analysis in whole blood or tissues.

Behavioral measures observed following the administration of CB1 agonists in mice were also evaluated to assess the in vivo effects of exposure to ‘Buzz’ smoke. The mouse tetrad test is a widely accepted measure of the cannabimimetic properties of CB1 agonists. The
tetrad reveals four psychotropic properties of Δ⁹-THC and other cannabinoids, including (1) antinociception, (2) catalepsy, (3) hypothermia, and (4) hypomobility (Martin et al., 1991). Hyperreflexia (Long et al., 2009; Patel and Hillard, 2001), straub tail (Cutler et al., 1975; Jacob et al., 1981) and ptosis (Aceto et al., 1998) have also been observed following the administration of CB1 agonists, and in particular following exposure to high doses of these compounds. Thus, mice were evaluated in two of the tetrad tests, hypothermia and catalepsy, as well as for hyperreflexia, straub tail and ptosis, to determine the cannabimimetic properties of ‘Buzz’ compound.

Materials and methods

Experimental

Six male C57BL/6 J mice (Jackson Laboratory, Bar Harbor, ME, USA) housed in the animal care quarters at Virginia Commonwealth University and maintained at 22 ± 2 °C on a 12 h light–dark cycle with food and water available ad libitum were brought to the test environment and allowed to acclimatize for 24 h. The animal study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The mice were exposed to the smoke of 200 mg of the burning HIP ‘Buzz’, containing 10.8 mg of JWH-018. The ‘Buzz’ was obtained from the internet (imzonged.com; TX, USA). Following exposure, the mice were assessed for hypothermia and catalepsy, which are indicative of CB1 activity (Little et al., 1988). Rectal temperatures taken with a digital thermometer (Fisher Scientific, Pittsburgh, PA, USA) at a depth of 2 cm and recorded to the nearest degree Centrigrade both before and 10 min after exposure. Mice were evaluated for catalepsy 15 min after exposure using the bar test as previously described (Cravatt et al., 2001; Wise et al., 2007). Briefly, the mouse was placed with its front paws on the bar that was 0.7 cm in diameter and 4.5 cm from the bench top. The time that the mouse remained immobile on the bar was recorded for a 10 s period. Twenty minutes after the exposure to the ‘Buzz’ smoke the mice were sacrificed. Immediately after death, blood, brain and liver were collected. The specimens were frozen at −80 °C until chemically analyzed.

Exposure apparatus

The exposure system used has been previously described (Lichtman et al., 2001). A 15 cm corncob pipe was used to burn 200 mg of the HIP. The smoke from the pipe was drawn through a 27.5 cm length of tygon tubing to the manifold at a flow rate of 552 mL/min using a vacuum pump and a flow regulator. A solenoid puffing device was used to alternate the flow of smoke and fresh air to the animals every 8 s. Tygon tubing, containing 0.5 g of glass-wool fiber to sequester the smoke, was connected to the exhaust of the manifold. The mice were placed into holding tubes that fit snugly into the manifold, consisting of six ports for a nose-only exposure. The mice were exposed to the smoke until the HIP was completely consumed, which occurred within a 2 min time period. If the material ceased to burn at any time, it was relit until completely consumed.

The glass-wool fibers connected to the exhaust manifold were collected after the exposure and stored in 5.0 mL of methanol for one week. One-hundred micro liters of the methanol extract were combined with 100 mL of the internal standard, dried under nitrogen, reconstituted with mobile phase, transferred to auto-sampler vials and analyzed using the presented HPLC/MS/MS method. The glass wool contained 235 mg JWH-018, 1 mg JWH-073 and 2.7 mg JWH-398.

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Reagents and supplies

The d$_3$-$\Delta^9$-tetrahydrocannabinol (d$_3$-THC) was purchased from Cerilliant (Texas, USA). Naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018), Fig. 1, was obtained from John W. Huffman (Clemson University). The methanol, acetonitrile, water and ammonium formate were purchased from Fisher Scientific (New Jersey, USA) and were HPLC-grade or better. A 0.1 mg/L JWH-018 working standard solution was prepared in methanol. A 0.5 mg/L d$_3$-THC working internal standard solution was also prepared in methanol. A negative control and eight-point calibration curves at concentrations of 1, 2.5, 5, 10, 20, 50, 100 and 200 ng/mL JWH-018 in 1.0 mL of drug-free whole blood were prepared with each analytical run.

Determination of JWH-018 in ‘Buzz’

The cannabimimetic content of the HIP, ‘Buzz’, was determined by continuous extraction of 50 mg of ‘Buzz’ with methanol for 72 h. A 100 mL aliquot of the extract was mixed with 20 mL of d$_3$-THC internal standard and then dried under dry nitrogen. The resultant residue was dissolved in 100 mL mobile phase and then analyzed using the instrument method described herein, but applying nonextracted calibrators. ‘Buzz’ was found to contain 5.4% JWH-018. Two other cannabimimetics were detected at less than 0.1%, JWH-073 and JWH-398 (w/w).

Extraction of blood and tissues

The extractions were performed using cold acetonitrile procedure as previously described for analysis of natural cannabinoids in blood (Poklis et al., 2010). Prior to extraction, 250 mL of mouse blood was diluted with 750 mL of drug-free human blood to create a sample size of 1 mL. The brains were homogenized by hand using a glass homogenizer with deionized water at a 1:4 dilution (w/w). Half of the resulting homogenate by mass was extracted according to the procedure. Added to each sample was 50 mL of the working internal standard containing 25 ng/mL of d$_3$-THC. These samples were mixed and allowed to equilibrate overnight. The following day 2mL of ice-cold acetonitrile was added drop by drop to each sample while vortexing. The samples were then centrifuged at 3500 rpm for 10 min. After centrifuging, the samples were placed in a −40 °C freezer for at least 2 h. The top layer of each sample containing the acetonitrile was then removed as completely as possible, making sure not to remove any of the aqueous layer, via a disposable glass pipette and placed in clean test tubes. Extracts were then dried using a Savant AES1000. The resultant residues were reconstituted with 100 mL of mobile phase and placed in autosampler vials for LC/MS/MS analysis.

Instrumental analysis

The LC/MS/MS system used was an Applied Bio systems 3200 Q trap with a turbo V source for TurboLonSpray attached to a Shimadzu SCL HPLC system controlled by Analyst 1.4.2 software. The chromatographic separation was performed using a Zorbax eclipse XDB-C$_{18}$ column, 4.6 $\times$ 75 mm, 3.5 mm (Agilent Technologies, USA). The mobile phase contained water–acetonitrile (20:80 v:v) with 0.1 m$\text{M}$ ammonium formate and was delivered at a flow rate of 0.5 mL/min. The source temperature was set at 650 °C and had a curtain gas at a flow rate of 30 mL/min. The ionspray voltage was 5000 V, with ion source gases 1 and 2 having flow rates of 60 mL/min. The acquisition mode used was multiple reaction monitoring. Table 1 lists the transition ions monitored, the corresponding deprotonation and collision energies used for JWH-018 and d$_3$-THC, the internal standard. The chromatographic method resolved d$_3$-THC and JWH-018 (Fig. 2). The total run time for the analytical method was 12 min.
Preparation of calibration standards and quality control samples in blood

Drug-free blood (in-house certified) provided the matrix for the study. Appropriate volumes of the working solutions of JWH-018 were added to the blood to obtain calibration standards. An eight-point calibration curve of 1, 2.5, 5, 10, 20, 50, 100 and 200 ng/mL was prepared before each analytical run. Quality control samples of JWH-018 were prepared at 1.0 ng/mL for limit of quantitation (LOQ), 2.5 ng/mL for the low quality control (LQC), 20 ng/mL for the medium quality control (MQC) and 75 ng/mL for the high quality control (HQC). Upper limit of quantitation (ULQ) samples above the high calibration point were prepared at concentrations of 500 and 1000 ng/mL. Tissue quality control samples of JWH-018, 10 ng/g for the LQC, 80 ng/g for the MQC and 100 ng/g for the HQC were prepared using drug-free 1:4 (tissue: water) brain or liver homogenates. All QC samples were prepared as a single lot and were stored at −20 °C until analysis. Calibrators and QC samples were extracted using the same procedure as described for the blood and tissue samples.

HPLC/MS/MS validation

The method validation was performed as follows. Validation runs containing calibration standards, blank samples, blank sample with internal standard added and replicates of LOQ, LQC, MQC and HQC samples were prepared and run on three separate days.

Linearity, LOQ and ULQ—A linear regression of the ratio of the peak area response of JWH-018 and internal standard (d3-THC) vs concentration was determined. For each calibration curve, the back-calculated standard concentrations were within 15% deviation from the nominal value except the LOQ, where it could be within 20%. The correlation coefficient ($r^2$) for each curve was 0.995 or better. The LOQ of JWH-018 was administratively set at 1 ng/mL and had a response of at least five times the signal-to-noise ratio of the drug-free calibrator response. The ULQ samples of 500 and 1000 ng/mL of JWH-018 were prepared to evaluate accuracy above the highest calibrator (200 ng/mL). These controls were analyzed and determined to be within 15% deviation from the nominal. This demonstrated that, if test specimens obtained from animal exposures to JWH-018 exceeded the calibration, these values would still be within the linear range of the assay. The calibration could then be adjusted by the addition of a calibrator reflecting the ULQ values. However, no test specimen in this study exceeded the high calibrator.

Accuracy/bias and precision—Accuracy/bias and precision were determined for the prepared QC samples in three different validation runs for blood. The percentage accuracy/bias of the method was calculated as the ratio of the mean JWH-018 concentrations of six aliquots of each QC sample analyzed in the same batch of samples, to the target concentration of the QC samples times 100. The criteria for acceptable assay accuracy/bias were quantified results within ±20% of the target value of the prepared QC samples, while precision expressed as coefficient of variation (CV) did not exceed 15% (20% for LOQ). Both intra and inter-day accuracy and precision were determined as not exceeding a 15% CV and 20% for the LOQ, as shown in Table 2. Accuracy/bias and inter-day precision for tissue QC sample were evaluated with three aliquots of each control The criteria for acceptable assay accuracy/bias were quantified results within ±20% of the target value of the prepared QC samples and the precision expressed as coefficient of variation (CV) did not exceed 20%, as shown in Table 3.

Absolute recovery—The percentage recovery was determined by first extracting five drug-free blood or brain tissue samples and then reconstituting these extracts with HPLC mobile phase containing the target concentrations of 10 ng/mL or 40 ng/g. These samples nullified any matrix effects from interfering with the recovery studies. Then five extracted
samples of blood or tissue at 10 ng/mL or 40 ng/g were prepared. These samples were then analyzed by the HPLC/MS/MS method described herein. The absolute recovery of the assay was then determined by the ratio of the peak areas for five aliquots of 10 ng/mL blood or 40 ng/g brain tissue samples divided by the peak areas of the 10 ng/mL or 40 ng/g samples prepared in the HPLC mobile phase times 100. The results show 97% (CV = 11%) absolute recovery in blood and 117% (CV = 11%) absolute recovery in brain tissue.

Selectivity and matrix—Six different lots of cannabimimetic-free blood were used to assess selectivity. Each individual lot was analyzed with and without internal standard. No co-eluting peaks with JWH-018 or the internal standard (d3-THC) were detected for any the six lots of cannabimimetic-free blood. This ensured that the endogenous blood components do not interfere with the assay. Six different lots of cannabimimetic-free bloods were fortified with 4 ng/mL JWH-018 and analyzed in triplicate. Three different lots of cannabimimetic-free brain and liver were used to assess selectivity in tissue analysis. Each individual lot was analyzed with and without internal standard. No co-eluting peaks were detected in any of the brain or liver lots.

Carryover—Sample carryover was evaluated in each of the validation batches using two different procedures. For the carryover study, an upper limit of quantification blood control (ULQ) containing 1000 ng/mL and tissue ULQ containing 4000 ng/g of JWH-018 were prepared in drug-free blood, brain and liver samples. First, immediately following the injection of an extracted ULQ, a sample of drug-free control was injected. The rejection criterion for carryover was set at the detection of JWH-018 at a concentration <20% of the LQC (1 ng/mL for blood or 4 ng/g for brain and liver). JWH-018 was not detected in the drug-free specimens. An additional procedure to evaluate possible analyte carryover during batch analysis involved injecting an extracted HQC (75 ng/mL for blood and 300 ng/g for brain and liver) immediately followed by an LQC (2.5 ng/mL for blood and 10 ng/g for brain and liver). This analysis was repeated consecutively six times for blood and three times each for brain and liver. The rejection criterion for carryover was set at a JWH-018 concentration with a bias of less than 20% of the target value of the LQC. Lack of carryover was confirmed with a bias within 15% of the LQC concentration for blood (target, 2.5 ng/mL; actual, 2.1 ng/mL ± 0.1), brain (target, 10 ng/g; actual, 10.6 ± 1.5) and liver (target, 10 ng/mL; actual, 10.1 ± 0.7).

Stability—Stability experiments were performed in blood at three QC concentrations (2.5, 20 and 75 ng/mL) with six replicates at each concentration. The stability in blood was assessed during storage and after three freeze–thaw cycles at −20 °C with at least 24 h between cycles. These freeze–thaw QC samples were then run against freshly prepared calibration standards. The bench-top stability at room temperature was assessed in blood for 24 h to cover for the processing time of the samples including transportation. These samples were considered to be stable in blood and the JWH-018 concentrations were within 20% of the nominal concentration for the QC samples tested. The post-preparative stability or the autosampler stability was assessed from re-injection reproducibility after storage of the samples in the autosampler for 72 h at room temperature. JWH-018 samples showed between 45 and 30% degradation.

Results and discussion

The presented HPLC/MS/MS method for analysis of blood and tissue specimens applied an administrative LOQ of 1.0 ng/mL for JWH-018. Extracted samples were stored at −20 °C before injection and at 6 °C on the autosampler to minimize degradation of the JWH-018. The methods of Teske et al. (2010) and Sebastian et al. (2011), applied to serum analysis, were more sensitive, with LOQs of 0.5 and 0.1 ng/mL, respectively. However, blood and
tissues are far more complex matrices than serum from which to extract and purify lipid soluble compounds such as JWH-018. Therefore, it was deemed that the analytical parameters of the presented method were sufficient for the goals of this study.

The responses of the mice were consistent with CB1 activity. The body temperatures of the mice dropped 7.3±1.1 °C from the baseline measurements only 10 min post-exposure, as shown in Table 4. By comparison, the body temperatures of mice exposed to smoke from 200 mg of marijuana containing 3.5% THC had a decrease in body temperature of 3–4 °C (Lichtman et al., 2001). All mice were cataleptic for the 10 s period. The additional behaviors observed in all of the mice included hyperreflexia, straub tail and ptosis. Moreover, all mice exhibited hypomobility in their home cages, remaining essentially motionless with the exception of demonstrating hyperreflexia. These behavioral measures indicate that exposure to ‘Buzz’ smoke results in in vivo effects that are similar to THC and other CB1 receptor agonists.

The weight of individual mice and concentration of JWH-018 following inhalation exposure to ‘Buzz’ smoke and the blood and tissue concentrations of JWH-018 are presented in Table 5. Twenty minutes after exposure to ‘Buzz’ smoke, blood concentrations of JWH-018 ranged from 54 to 166 ng/mL (mean ± SD; 82 ± 42 ng/mL). Brain concentrations of JWH-018 ranged from 316 to 708 ng/g (mean ± SD; 510 ± 166 ng/g), while liver ranged from 1367 to 3219 ng/g (mean ± SD; 1990 ± 752 ng/g). The concentrations for one brain and one liver specimen were not determined owing to a laboratory error. The mean blood-to-brain ratio for JWH-018 was 6.8 and ranged from 4.2 to 10.9. The high concentration in brain compared with blood is due to the highly lipophilic nature of JWH-018. The presence of this cannabimimetic in brain and the hypothermic and cataleptic responses of the mice inhalation exposure of this drug are consistent with the hypothesis that the behavioral effects of ‘Buzz’ are attributable to JWH-018.

While it is difficult to make direct comparisons, it is interesting to note that the binding affinity (Kᵢ) of JWH-018 to the CB1 receptor is considerably higher than the affinity of THC (respective Kᵢ values of 9.5 and 41 nM; Wiley et al., 2008). Indeed these in vivo results in the presented study suggest that this HIP is highly potent and efficacious in producing cannabimimetic effect. Thus, recreational use of HIPs can result in exposure to a high dose of JWH-018 and strong cannabimimetic effects, which could account for the increase in emergency room visits by users (Simmons et al., 2011). In the same exposure system with marijuana containing approximately 4% THC, at 20 min post-exposure, the JWH-018 blood concentrations are much lower than THC, 200–300 ng/mL (Lichtman et al., 2001; Poklis et al., 2010), while the brain values are on average higher, 300–600 ng/g (Poklis et al., 2010; Varvel et al., 2006). More generally, the present study provides a reliable method to quantify JWH-018 and other increasingly abused synthetic cannabinoids from biological tissues.

Acknowledgments
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Abbreviations used
- CB1: cannabinoid receptor type 1
- HIP: herbal incense product
- THC: Δ⁹-tetrahydrocannabinol

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References


Figure 1.
Structures of JWH-018 and d₃-THC (IS).
Figure 2.
The chromatographic resolution of JWH-018 and d3-THC in a typical mouse blood specimen.
Table 1

JWH-018 and d3-THC (IS) transition ions, and corresponding deprotonation (DP) and collision energies (CE)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention times (min)</th>
<th>Transition ions</th>
<th>DP (V)</th>
<th>CE (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d3-THC</td>
<td>9</td>
<td>318 → 196</td>
<td>46</td>
<td>27</td>
</tr>
<tr>
<td>JWH-018</td>
<td>5.7</td>
<td>342 → 155</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>342 → 127</td>
<td>65</td>
<td>50</td>
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### Table 2

Accuracy/bias and precision

<table>
<thead>
<tr>
<th>Blood controls, ng/mL (%CV)</th>
<th>Intra-day ($n=6$)</th>
<th>Inter-day ($n=9$)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOQ (1.0 ng/mL)</td>
<td>0.88 (5.5)</td>
<td>0.98 (9.7)</td>
</tr>
<tr>
<td>Low QC (2.5 ng/mL)</td>
<td>2.2 (2.7)</td>
<td>2.4 (8.5)</td>
</tr>
<tr>
<td>Mid QC (20 ng/mL)</td>
<td>20.4 (4.6)</td>
<td>21.9 (5.3)</td>
</tr>
<tr>
<td>High QC (75 ng/mL)</td>
<td>81.4 (5.5)</td>
<td>82.7 (5.9)</td>
</tr>
</tbody>
</table>

LOQ, Limit of quantitation; QC, quality control.
Table 3

JWH-018 controls for brain and liver

<table>
<thead>
<tr>
<th>Tissue controls, ng/g (%CV)</th>
<th>Control</th>
<th>Brain (n = 3)</th>
<th>Liver (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low QC (10 ng/g)</td>
<td>10.6 (7.0)</td>
<td>10.1 (13)</td>
<td></td>
</tr>
<tr>
<td>Mid QC (80 ng/g)</td>
<td>86.4 (17)</td>
<td>85.4 (17)</td>
<td></td>
</tr>
<tr>
<td>High QC (300 ng/g)</td>
<td>317 (17)</td>
<td>333 (8.2)</td>
<td></td>
</tr>
</tbody>
</table>

QC, Quality control.
### Table 4

Effect of ‘Buzz’ smoke exposure on core body temperature

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Pre (°C)</th>
<th>Post (°C)</th>
<th>Δ (°C)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>30</td>
<td>−7</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>30</td>
<td>−7</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
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<td>29</td>
<td>−8</td>
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<tr>
<td>Mean</td>
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<td>30.0</td>
<td>−7.3</td>
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<td>SD</td>
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</tr>
<tr>
<td>%CV</td>
<td>1.2</td>
<td>3.3</td>
<td>−15.2</td>
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Table 5

Mouse weights and JWH-018 concentration in blood, brain and liver

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Weight (g)</th>
<th>Blood (ng/mL)</th>
<th>Brain (ng/g)</th>
<th>Liver (ng/g)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>77</td>
<td>ND</td>
<td>1367</td>
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<td>1706</td>
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<td>166</td>
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<td>2171</td>
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<td>ND</td>
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<tr>
<td>Mean</td>
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<td>82</td>
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ND, Not determined.