Journal of Pharmaceutical and Biomedical Analysis 140 (2017) 215–222

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

# Pharmacokinetic properties of the synthetic cannabinoid JWH-018 and of its metabolites in serum after inhalation

Stefan W. Toennes [a,*], Anna Geraths [a], Werner Pogoda [a], Alexander Paulke [a], Cora Wunder [a], Eef L. Theunissen [b], Johannes G. Ramaekers [b]

[a] Institute of Legal Medicine, Goethe-University Frankfurt, Kennedyallee 104, D-60596 Frankfurt/Main, Germany
[b] Department Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands

ARTICLE INFO

*Article history:*
Received 30 January 2017
Received in revised form 14 March 2017
Accepted 20 March 2017
Available online 24 March 2017

*Keywords:*
Pharmacokinetics
Synthetic cannabinoids
JWH-018
Metabolites
Liquid chromatography–mass spectrometry

ABSTRACT

Each year, synthetic cannabinoids are occurring in high numbers in the illicit drug market, but data on their pharmacology and toxicology are scarcely available. Therefore, a pilot study was performed to assess adverse effects of JWH-018, which is one of the oldest and best known synthetic cannabinoids.

Six subjects inhaled smoke from 2 and 3 mg JWH-018. The drug and nine of its metabolites were analyzed in their blood samples taken during the following 12 h by liquid chromatography–mass spectrometry (LC–MSMS).

The maximum concentration of JWH-018 reached 2.9–9.9 ng/ml after inhalation and markedly decreased during the next 1.5 h, followed by a multiexponential decline ($t_{1/2}$ in median 1.3 h and 5.7 h). The concentration of the pentanoic acid metabolite was slightly higher than that of the 3-, 4- and 5-hydroxypentyl metabolites and of the 6-hydroxyindol metabolite. The data also suggest a multiexponential decline and slow terminal elimination of JWH-018 and all metabolites.

The detection of JWH-018 and of its metabolites in serum requires high analytical sensitivity. The pharmacokinetic properties of inhaled JWH-018 are similar to that of THC. A slow terminal elimination of drug and metabolites may lead to accumulation in chronic users.



## 1. Introduction

New synthetic cannabinoids are still emerging in high numbers [1]. However, due to their potential to induce severe adverse reactions [2], no systematic studies have been performed so far. In order to assess the adverse effects of synthetic cannabinoids, a study with a small number of subjects using a low dose of the well-known compound, JWH-018, was performed. One aspect of the study was the investigation of the pharmacokinetic properties of JWH-018. Some reports on the detection of the drug in human blood or serum describe some data on the concentration–time course of JWH-018 [3,4], others on JWH-018 concentrations measured in hospitalized patients as well as in forensic and postmortem cases [5–11]. Only one study included two of the drug's metabolites, JWH-018 pentanoic acid and JWH-018 N-(5-hydroxypentyl) [10].

In the present study, the pharmacokinetic properties of JWH-018 are described using serum samples collected up to 12 h after inhaling a 2 or 3 mg dose of the cannabinoid. Since metabolites also exhibit agonistic activity [12], an analytical procedure was developed to assay nine commercially available metabolites.

## 2. Experimental

### 2.1. Chemicals and reference standards

Reference substance of JWH-018, JWH-018 N-(2-hydroxypentyl) metabolite (2-HOpentyl-JWH-018), JWH-018 N-(3-hydroxypentyl) metabolite (3-HOpentyl-JWH-018) and JWH-018 N-(4-hydroxypentyl) metabolite (4-HOpentyl-JWH-018) were purchased from Lipomed AG (Arlesheim, Switzerland). JWH-018 N-(5-hydroxypentyl) metabolite (5-HOpentyl-JWH-018), JWH-018 4-hydroxyindole metabolite (4-HOindol-JWH-018), JWH-018 5-hydroxyindole metabolite (5-HOindol-JWH-018), JWH-018 6-hydroxyindole metabolite (6-HOindol-JWH-018), JWH-018 7-hydroxyindole metabolite (7-HOindol-JWH-018), JWH-018 N-pentanoic acid metabolite (JWH-018 pentanoic acid) as well as the internal standards JWH-018-$d_{11}$, JWH-018 pentanoic acid metabolite-d4, JWH-018 N-(5-hydroxypentyl) metabolite-$d_5$

* Corresponding author.
*E-mail address:* toennes@em.uni-frankfurt.de (S.W. Toennes).

http://dx.doi.org/10.1016/j.jpba.2017.03.043
0731-7085/

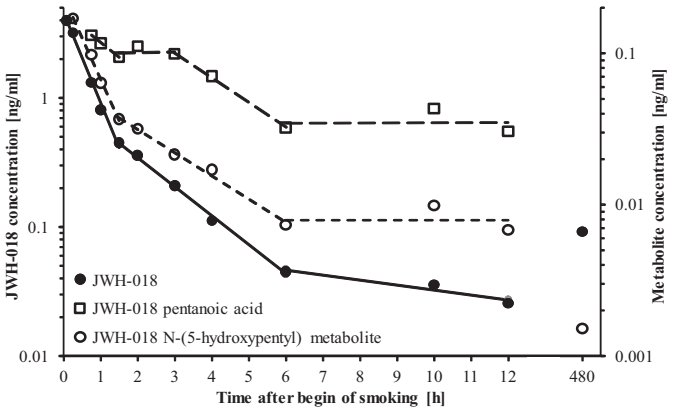


**Fig. 1.** Typical concentration–time data (subject #7 after inhaling 2 mg JWH-018) of JWH-018 and of the metabolites 5-HOpentyl-JWH-018 and JWH-018 pentanoic acid in logarithmic scale (concentrations of JWH-018 refer to the left *y*-axis, those of the two metabolites to the right *y*-axis). The lines indicate the exponential regression analysis results in different distribution/elimination phases. The concentrations of JWH-018 and 5-HOpentyl-JWH-018 in the baseline sample prior to inhaling the 3 mg dose, 20 days (480 h) after the 2 mg dose, are also given (cf. Fig. 3).

and JWH-018 4-hydroxyindole metabolite-$d_9$ were obtained from Cayman Chemical Company (Ann Arbor, USA).

LC-grade water was from LGC Promochem (Wesel, Germany) and LC-grade acetonitrile from Karl Roth GmbH (Karlsruhe, Germany). All other chemicals or solvents were obtained from Sigma–Aldrich (Munich, Germany) and were of analytical or LC grade. Drug free serum was provided by the clinic's blood bank and was tested for the absence of drugs of abuse and medical drugs.

### 2.2. Biological samples

A placebo controlled, three-way cross-over study was conducted in 6 healthy regular users of cannabis (2 males, 4 females; average age 23.5 years). On two occasions, each subject inhaled the smoke of a 2 mg and 3 mg dose of JWH-018. The study was approved by the Medical Ethics Committee of Maastricht University.

JWH-018 powder was mixed with a small amount of plant material, and heated in a 10 cm glass pipe ('crack pipe'). While the bowl of the pipe contained the compound mixture, a 40 cm plastic tube was connected to the end of the pipe. After closing the air holes, the bowl was heated for about 15 s. When a smoke was formed, the air holes were opened and the subject was instructed to immediately inhale the smoke at once via the plastic tube. Thus, substance application lasted less than a minute.

Blood samples were taken prior to drug application (baseline sample) and 5 and 15 min, 1, 1.5, 2, 3, 4, 6, 10 and 12 h after the inhalation. Blood samples were centrifuged and stored at −18 °C until analysis for JWH-018 and its metabolites as described below.

### 2.3. Evaluation of the data

The quantitative data were evaluated as described previously [13,14]. For every subject and analyte, phases of linear decline of the logarithmic concentration–time curves were identified (cf. Fig. 1). The apparent elimination half-lives ($t_{½}$) were calculated from the results of exponential regression analysis of the data. The areas under the curves (AUC) were estimated using the trapezoidal rule.

### 2.4. Sample preparation and extraction procedure

Serum (0.5 ml) was extracted after the addition of 1 ml of phosphate buffer (0.5 M, pH 7), 50 μl of internal standard solution (a mixture containing 0.02 ng/μl JWH-018-$d_{11}$, JWH-018 pentanoic acid-$d_4$, 5-HOpentyl-JWH-018-$d_5$ and 4-HOindol-JWH-018-$d_9$ in methanol) and 3 ml of tert-butylmethylether/*n*-hexane/ethyl acetate (45:45:10, v/v/v). After two minutes of mixing and centrifugation at 13,000 × *g* the upper organic layer was transferred to a silanized glass tube and evaporated to dryness at 25 °C with a stream of air. The dry residue was reconstituted with 50 μl of acetonitrile/methanol/water (6:6:4, v/v/v).

### 2.5. Calibration standards and quality controls

Calibration standards were prepared spiking 0.5 ml of drug free serum with diluted methanolic standard solutions, which contained a mixture of JWH-018, 2-HOpentyl-JWH-018, 3-HOpentyl-JWH-018, 4-HOpentyl-JWH-018, 5-HOpentyl-JWH-018, 4-HOindol-JWH-018, 5-HOindol-JWH-018, 6-HOindol-JWH-018, 7-HOindol-JWH-018 and JWH-018 pentanoic acid. The final concentrations of the calibrators were 0.005, 0.01, 0.025, 0.1, 0.5, 1, 5 and 10 ng/ml.

Three levels of quality control samples (QC) were also prepared using 0.5 ml of drug free serum. The concentrations of low-, medium-, and high QC samples were: 0.5, 5 and 10 ng/ml. Calibration standards and quality control samples were processed as described above.

### 2.6. LC–MS/MS instrumentation and analytical conditions

For analysis a LC–MS/MS system from Agilent (Waldbronn, Germany) was used consisting of a 1290 Infinity LC coupled via JetStream electrospray interface (ESI) to a 6460 Triple Quadrupole Mass Spectrometer. After injection of 3 μl of extracts, analytes were separated at 50 °C on an Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 μm LC column. The mobile phase consisted of water containing 5 mM ammonium formate and 0.01% formic acid (A) and acetonitrile containing 0.01% formic acid (B). The elution program started with 55% B at a flow rate of 0.7 ml/min, increased during 4 min to 65%, then during 2 min to 70%, during 0.5 min to 75%, during 1.5 min to 85% and was then increased to 90% which was held for 1 min followed by re-equilibration for 2 min.

Electrospray parameters were as follows: gas flow 13 l/min (350 °C); nebulizer 60 psi; sheath gas flow 12 l/min (350 °C); capillary voltage 3500 V. The MS/MS was operated in multiple reaction monitoring mode (MRM) with one transition for internal standards and two transitions recorded for each analyte. In time segment 1 (start time 0.2 min) the transitions were (*m/z*, collision energy in parentheses, quantifier underlined): JWH-018 pentanoic acid-$d_4$ 376.2 → 155 (20); JWH-018 pentanoic acid 372.2 → 155 (20), 372.2 → 127 (52); 5-HOpentyl-JWH-018-$d_5$ 363.2 → 127 (52); all JWH-018 N-hydroxypentyl metabolites: 358.2 → 155 (20), 358.2 → 127 (20). In time segment 2 (start time 5.5 min) the transitions were: JWH-018-$d_{11}$ 353.3 → 225 (20); JWH-018 342.2 → 155 (20), 342.2 → 127 (48); 4-HOindol-JWH-018-$d_9$ 367.2 → 239 (24); all JWH-018 hydroxyindole metabolites: 358.2 → 155 (24), 358.2 → 127 (52).

Data evaluation was performed using the Agilent MassHunter Software (B.07.00). For identification a deviation of ±0.1 min of the expected retention time compared to calibrators, and a quantifier/qualifier ratio within 20% of the ratio measured in calibrators were required.

### 2.7. Method validation

Validation was performed according to current guidelines [15,16]. For statistical evaluation Valistat 2.0 software (Arvecon GmbH, Walldorf, Germany) was used. Selectivity was assessed with drug free human serum samples from different donors ($n = 10$) with and without addition of internal standards (blank and zero