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Gas chromatography–mass spectrometry of JWH-018 metabolites in urine samples with direct comparison to analytical standards

Beth Emerson [a], Bill Durham [a,*], Jennifer Gidden [b], Jackson O. Lay Jr. [b]

[a] Department of Chemistry, University of Arkansas, Fayetteville, AR 72701, United States
[b] Arkansas Statewide Mass Spectrometry Facility, Fayetteville, AR 72701, United States

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ABSTRACT

JWH-018 (1-pentyl-3-(1-naphthoyl)indole) is one of numerous potential aminoalkylindoles contained in products marketed as 'K2' or 'Spice'. Investigation of the urinary metabolites from consumption of these compounds is important because they are banned in the United States and many European countries. An efficient extraction procedure and gas chromatography–mass spectrometry (GC–MS) method were developed for detection of 'K2' metabolites in urine from individuals suspected of using these products. Analytical standards were used to elucidate the structure-specific mass spectral fragmentations and retention properties to confirm proposed identifications and support quantitative studies. A procedure for the synthesis of one of these metabolites (5-hydroxypentyl JWH-018) was also developed. Results are comparable to existing LC–MS/MS methods, with the same primary metabolites detected. The specific metabolite hydrolysis products include 4-hydroxpentyl, 5-hydroxypentyl, and N-pentanoic acid derivatives.

1. Introduction

Since 2004, herbal mixtures under the brand names 'Spice', 'K2', and others have been sold via the internet and in 'headshops'. Although these products are marketed as incense, the blends have been smoked in a manner similar to tobacco products giving users cannabis-like effects comparable to marijuana [1,2]. These psychoactive effects are a result of synthetic cannabinoids, including aminoalkylindole (AAI) and cyclohexylphenol (CP) compounds, added to the mixtures. One of the first and most commonly reported additives is JWH-018, an AAI with binding affinity to the CB_1 and CB_2 cannabinoid receptors [1–3].

Analysis of synthetic cannabinoids is relevant from both a clinical and a law enforcement perspective. Several studies have investigated their detection in seized material and in bodily fluids [1,3–11]. Reliable detection of AAIs and metabolites in a variety of substrates is critical because the numbers of severe episodes of intoxication are increasing at healthcare facilities. In addition, five synthetic cannabinoids (JWH-018, JWH-073, JWH-200, CP-47,497, and cannabicyclohexanol) have been classified as Schedule 1 substances by the U.S. Drug Enforcement Administration (DEA) [12,13]. These compounds, and other JWH analogs, are also banned in many European countries [14].

Several methods have been reported for detection of JWH metabolites in urine samples using liquid chromatography and tandem mass spectrometry (LC–MS/MS) [6–9]. The development of a second technique, using a different approach, would be useful for confirmation. We elected to develop a GC–MS method to complement the existing LC–MS approach because it has higher specificity in both the chromatographic and the mass spectral detection steps, the obtained electron impact (EI) spectra are reproducible allowing them to be searched and matched in a library database, and the instrument is usually less expensive [15,16]. The GC chromatographic column provides an order of magnitude more theoretical plates for separation, and the mass spectral ionization step (EI) produces suppression-free spectra with higher structure specific information [15–17]. The advantage of the LC–MS/MS technique is that it is uniquely amenable to direct analysis of aqueous solutions and is better suited for non-volatile compounds [15,17,18]. With suitable extraction of the problematic components beforehand, GC–MS should provide a powerful and complementary approach for the characterization of these metabolites after separation from the urine matrix. This approach has not yet been extensively studied due to a lack of method validation studies (i.e. detection limits, recovery efficiency, and quantification) and the difficulty in obtaining proper analytical

* Corresponding author at: Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701, United States.
Tel.: +1 479 575 7945; fax: +1 479 575 4049.
E-mail address: bdurham@uark.edu (B. Durham).

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standards [10,11]. These limitations permit only tentative or semi-quantitative assignments of suspected urinary metabolites by GC–MS. Additionally, identification of some metabolites is difficult because of the presence of isomeric compounds with similar mass spectral fragmentation patterns and retention properties. This can lead to differences in the reported identity of metabolites from the same parent compounds. For example, the main urinary metabolites of JWH-018 reported by Sobolevksy et al. involve monohydroxylation on the indole ring [10] whereas Grigoryev et al. identify monohydroxylation on the pentyl chain [11]. The synthesis of the appropriate analytical standards is probably the best approach to resolve such differences. Even with standards, however, differences for the main metabolites and their relative abundance have been reported by LC–MS/MS. Chimalakonda et al. identified three metabolites with the following abundance ranking: 4-hydroxypentyl > 5-hydroxypentyl > N-pentanoic acid derivatives [6]. Analysis of urine samples with a similar method by ElSohly et al. identified three main metabolites with the order as N-pentanoic acid > 5-hydroxypentyl > 6-hydroxyindole derivatives [19].

The aim of this present work was to develop a GC–MS method that is analogous and complementary to the existing LC–MS/MS method. A GC–MS method would be of significant value for confirmation of LC–MS/MS results with regard to the main urinary metabolites of JWH-018. This is especially important given the differences between detected metabolites from different studies as described above. Development of a GC–MS method also provides an alternative technology that may be better suited to the existing equipment in a particular laboratory. GC–MS is a fundamental tool in forensic toxicology and was the only permitted method for urine drug testing from 1988 to October 2010 under the Mandatory Guidelines for Federal Workplace Drug Testing Programs. New guidelines now permit alternative technologies (LC–MS, GC–MS/MS, and LC–MS/MS) to be used as long as the methods are scientifically validated [20–22]. In this current report, three metabolite hydrolysis products (4-hydroxypentyl, 5-hydroxypentyl, and a carboxylated derivative of JWH-018) and the native compound of JWH-018 were used as reference standards for comparison to urine samples from suspected 'K2' users. These particular metabolites were chosen because of their reported abundance in urine samples from 'K2' users [6–9]. A method meeting criteria established for proof of identity was developed based on chromatographic retention properties and monitoring four diagnostic ions for each compound [23,24]. The efficiency of extracting the metabolites from urine samples using solid phase extraction (SPE) and calculation of detection limits were also monitored.

2. Materials and methods

2.1. Reagents

The reagents and solvents were obtained from EMD chemicals (Gibbstown, NJ), Sigma Aldrich (St. Louis, MO), and TCI America (Portland, OR). N,O-Bis(trimethylsilyl) trifluoroacetamide + 10% trimethylchlorosilane (BSTFA + 10% TMCS) was purchased from Regis Technologies (Morton Grove, IL). SPE disposable cartridges (octadecyl C18) were manufactured by J.T. Baker (Phillipsburg, NJ). A certified negative control urine sample was obtained from Biochemical Diagnostics Inc. (Edgewood, NY). Because of the lack of availability at the time of analysis, the 5-hydroxypentyl JWH-0108 metabolite was synthesized as reported below. As the study progressed, two additional standards (4-hydroxypentyl and N-pentanoic acid JWH-018 derivatives) were made commercially available by Cayman Chemical (Ann Arbor, MI). JWH-018 was synthesized as reported in the literature [25].

2.2. Synthesis of 5-hydroxypentyl JWH-018 metabolite

This JWH-018 metabolite was synthesized as indicated in Scheme 1: the hydroxyl group of 5-bromopentanol (**1**) was protected with tert-butyldimethylsilyl chloride (TBDMSCl) to give product **2** [26]. A nucleophilic substitution reaction was used to obtain product **3** by reaction of the protected bromopentane with indole [27]. Friedel–Crafts acylation of the protected pentylindole with 1-naphthoyl chloride and Me_2AlCl afforded product **4** [25]. Deprotection of product **4** with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) produced product **5** in 71% yield [26]. All molecular structures were confirmed using GC–MS and ^1H NMR. Conditions for GC–MS analysis are discussed below. ^1H NMR spectra were recorded on a Bruker 300 MHz spectrometer using $CDCl_3$ as the solvent with TMS as the internal standard.

2.2.1. 5-Bromopentoxy-tert-butyl-dimethyl-silane

To a solution of imidazole (1.2 g, 17.6 mmol) in 10 mL DMF was added 5-bromopentan-1-ol (**1**) (2.0 g, 12.0 mmol) and 1 M TBDMSCl in THF (15 mL, 15 mmol) at 0 °C under N_2. Stirring was continued overnight at room temperature. The mixture was diluted with Et_2O and washed with 1 N HCl, water, and brine. The filtrate was then dried with Na_2SO_4 and concentrated. Product **2** (2.4 g, 71%) was afforded after purification by silica gel column chromatography (petroleum ether/Et_2O, 50:1) as a colorless oil [26].

2.2.2. Tert-butyl-(5-indol-1-ylpentoxy)-dimethyl-silane

To a solution of **2** (8.4 g, 30 mmol) in 50 mL DMF was added indole (1.2 g, 10.2 mmol) and ground KOH powder (0.6 g, 10.7 mmol). The mixture was stirred overnight at room temperature. Water (100 mL) was added, and the product was extracted into ether (3× 50 mL). The ether extracts were washed with water and dried with $MgSO_4$. After concentrating the solution, product **3** (2.0 g, 62%) was isolated through chromatography (petroleum ether followed by petroleum ether/ether, 10:1) as an oil [27].

2.2.3. [1-[5-[Tert-butyl(dimethyl)silyl]oxypentyl]indol-3-yl]-(1-naphthyl)methanone

To a stirred solution of **3** (0.16 g, 0.50 mmol) in 1.5 mL dry CH_2Cl_2 at 0 °C under N_2 was added dropwise Me_2AlCl (1 M in hexanes, 0.75 mL, 0.75 mmol). After stirring the mixture for 30 min at 0 °C, 1-naphthoyl chloride (0.12 g, 0.63 mmol) in 1.5 mL of CH_2Cl_2 was added. The reaction mixture was stirred at 0 °C until the reaction was complete as indicated by thin layer chromatography (TLC) analysis (approximately 1 h). The mixture was poured into iced 1 M aqueous HCl and extracted with CH_2Cl_2 (3× 50 mL). The extracts were washed with aqueous $NaHCO_3$ and then dried with $MgSO_4$. After evaporation of the solvent, chromatography (petroleum ether/ethyl acetate, 9:1) was used to obtain product **4** (0.17 g, 72%) as an off-white solid [25].

2.2.4. [1-(5-Hydropentyl)indol-3-yl]-(1-naphthyl)methanone

To a solution of **4** (4.3 g, 9.1 mmol) in 10 mL of THF was added 1 M TBAF in THF (18.4 mL, 18.4 mmol). The reaction mixture was stirred for 2 h and then quenched with MeOH. The mixture was washed with water and brine then dried with Na_2SO_4. After concentrating the solution, chromatography was used to give product **5** (2.3 g, 71%) as an off-white solid [26].

2.3. Urine samples of suspected 'K2' users

The urine samples analyzed in this study were collected from three individuals through a drug testing program operated by Employee Screening Management (Fayetteville, AR). No information regarding prior drug history or admittance to smoking any 'K2' products of the participants was provided. Samples were supplied with an assigned number with no personal information exchanged. Samples were first analyzed by the Arkansas Department of Health, Public Health Laboratory using their established LC–MS/MS method [7].

2.4. Preparation of urine samples

A 1-mL urine sample containing an internal standard of bisphenol A (BPA, 900 ng/mL) was evaporated to ~0.25 mL under N_2 at room temperature. Hydrolysis of glucuronic acid conjugates was completed by addition of 0.5 mL trifluoroacetic acid (TFA) to the dried residue and heating the sample for 40 min at 100 °C. After cooling to room temperature, ammonium hydroxide (~4.5 mL, 28–30%) was added to adjust the pH to ~9 as monitored with pH paper.

SPE cartridges (C18) were conditioned prior to analysis by rinsing the column with 3 mL methanol followed by 10 mL distilled water. The urine sample, prepared as described above, was then passed through the cartridge followed by rinsing with 10 mL distilled water. After discarding the above washing solutions, the analytes of interest were eluted with 4 mL methanol. The eluted methanol was evaporated to dryness at 60 °C (Centrivap Concentrator, Labconco, Kansas City, MO). The residue was then dissolved in 150 μL DMF and derivatized by addition of 150 μL BSTFA + 10% TMCS. After heating for 25 min at 70 °C, the samples were analyzed by GC/MS. To avoid contamination, a separate SPE cartridge was used for each sample.

2.5. Method parameters

2.5.1. Specificity

Five urine samples, collected over five consecutive days, were obtained from a healthy individual and prepared as described above. Samples were analyzed using