Journal of Chromatography B, 875 (2008) 465–470

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous analysis of THC and its metabolites in blood using liquid chromatography–tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 20 May 2008
Accepted 28 September 2008
Available online 2 October 2008

Keywords:
THC
Blood
LLE
LC–MS/MS

ABSTRACT

Cannabis is considered to be the most widely abused illicit drug in Europe. Consequently, sensitive and specific analytical methods are needed for forensic purposes and for cannabinoid pharmacokinetic and pharmacodynamic studies. A simple, rapid and highly sensitive and specific method for the extraction and quantification of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy- Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy- Δ^9-tetrahydrocannabinol (THC-COOH) in blood is presented. The method was fully validated according to international guidelines and comprises simultaneous liquid–liquid extraction (LLE) of the three analytes with hexane:ethyl acetate (90:10, v/v) into a single eluant followed by separation and quantification using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Chromatographic separation was achieved using a XBridge C_{18} column eluted isocratically with methanol:0.1% formic acid (80:20, v/v). Selectivity of the method was achieved by a combination of retention time, and two precursor–product ion transitions. The use of the LLE was demonstrated to be highly effective and led to significant decreases in the interferences present in the matrix. Validation of the method was performed using 250 μL of blood. The method was linear over the range investigated (0.5–40 μg/L for THC, 1–40 μg/L for 11-OH-THC, and 2–160 μg/L for THC-COOH) with excellent intra-assay and inter-assay precision; relative standard deviations (RSDs) were <12% for THC and 11-OH-THC and <8% for THC-COOH for certified quality control samples. The lower limit of quantification was fixed at the lowest calibrator in the linearity experiments. No instability was observed after repeated freezing and thawing or in processed samples. The method was subsequently applied to 63 authentic blood samples obtained from toxicology cases. The validation and actual sample analysis results show that this method is rugged, precise, accurate, and well suited for routine analysis.

1. Introduction

Cannabis is considered to be the most widely abused illicit drug in Europe. Indeed, statistical information shows that 30% of the under-forties age group have already consumed this drug [1,2].

Δ^9-Tetrahydrocannabinol (THC) is the main psychoactive constituent. During marijuana smoking, THC is rapidly absorbed in larger amounts than when taken orally and, due to its strong lypophilic nature, it spreads rapidly throughout the body. It is mainly metabolized to 11-hydroxy- Δ^9-tetrahydrocannabinol (11-OH-THC) by the human body. This metabolite is still psychoactive and is further oxidized to 11-nor-9-carboxy- Δ^9-tetrahydrocannabinol (THC-COOH). In humans and animals more than 100 metabolites could be identified but 11-OH-THC and THC-COOH are the most predominant. Metabolism mainly occurs in the liver by cytochrome P450 enzymes CYP2C9, CYP2C19 and CYP3A4 [3].

Urine drug concentration data do not provide adequate answers to demanding clinical and forensic questions. These are more readily answered with quantitative blood data which provides more information related to the current state of impairment. However, the analysis of blood can be more challenging due to the presence of lipophilic and proteinaceous compounds not usually found in urine, the need for substantially lower sensitivity limits and the lower sample volume available.

Due to the high specificity and the increased signal-to-noise in combination with short chromatographic run times, liquid

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doi:10.1016/j.jchromb.2008.09.032

chromatography–tandem mass spectrometry (LC–MS/MS) allows for specific, selective and sensitive analysis of compounds with a wide polarity range in samples of various nature. It offers the possibility to simplify sample preparation, although this approach should be treated with caution due to the possibility of ion suppression or enhancement as a result of the matrix. Consequently, attention must be paid to the choice of the sampling method and the influence of the collected matrix on the LC–MS/MS analysis.

Several methods have been described for the quantification of THC and its metabolites in blood. Immunochemical methods, mainly ELISA, are generally used as screening tools for cannabis use [4]. However, for workplace drug testing, driving under the influence of drugs and for forensic cases, the confirmation of positive immunoassay test results is necessary. It is usually performed by gas chromatography–mass spectrometry (GC–MS) methodologies [5–7]. However, GC requires time-consuming sample preparation and the need to use various derivatization techniques. In contrast to GC, no decomposition of the cannabinoids occurs during liquid chromatography and hence the cannabinoid acid forms may be analyzed directly. Several papers report the application of LC–MS(/MS) [8–14]. Most of them require high sample volume (1 mL) to achieve high sensitivity, they are focused on just one compound and/or the method is not fully validated (following all criteria for chromatographic assays). All of these aspects are significant at the moment of development of a method since (a) the amount of sample required is an analytical limitation, (b) forensic toxicologist may be required to analyze THC and other hydroxylated metabolites in blood to evaluate an impairment status and (c) complete validation is required to guarantee the robustness of the method.

Our aim was to develop and fully validate a simple, fast and sensitive LC–MS/MS method for the confirmation of THC, 11-OH-THC and THC-COOH in blood which required only a minimal volume of sample and with an efficient sample clean-up procedure.

2. Experimental

2.1. Chemicals

Individual stock solutions of THC, 11-OH-THC and THC-COOH (all certified at a concentration of 1 mg/mL in methanol), and the internal standards (I.S.) [$^2H_3$]THC (THC-d_3), [$^2H_3$]11-OH-THC (11-OH-THC-d_3) and [$^2H_9$]THC-COOH (THC-COOH-d_9) (certified concentration of 0.1 mg/mL in methanol) were obtained from LGC Promochem (Molsheim, France).

Methanol (LC–MS grade), 0.1% formic acid in water (UPLC grade) and water (HPLC grade) were purchased from Biosolve (Valkenswaard, The Netherlands). N-hexane (for chromatography), ethylacetate (for chromatography) and acetic acid (glacial) were obtained from Merck (Darmstadt, Germany).

External quality control (QC) samples were obtained from Medichem World (Steinenbronn, Germany).

2.2. Specimens

Pooled blank blood samples were used for development and validation of the procedure and were obtained from a local blood bank. Authentic samples were obtained from toxicology cases.

2.3. Preparation of standard solutions and sample extraction

Separate working solutions of the drugs, for tuning and selectivity experiments, were prepared in the laboratory at a concentration of 4 mg/L in methanol. A mixed working solution of non-deuterated compounds at 4 mg/L for THC and 11-OH-THC and of 16 mg/L for THC-COOH, in methanol was used for the preparation of calibrators. A mixed I.S. working solution of 1 mg/L for THC and 11-OH-THC and of 4 mg/L for THC-COOH, was prepared in methanol. Working solutions were stored at 4 °C, and were prepared monthly.

To obtain the lower concentrations needed for internal standardization and validation of each experiment, further dilutions in methanol were prepared the same day.

The extraction procedure was carried out in 10 mL disposable screw top vials of high quality glassware (Chromacol, Herts, UK) using 250 μL of blood. Fifty microliters of the I.S. working solution, 750 μL of deionised water and 200 μL of 10% acetic acid (glacial) were added. After adding 4 mL of hexane:ethyl acetate (90:10, v/v) mechanical shaking was carried out for 30 min. Then, the samples were centrifuged (10 min at 3000 × *g*), the organic phase was transferred to a 5 mL disposable screw top vial (Chromacol) and then evaporated to dryness with a vacuum centrifuge (Jouan, Saint Herblain, France). The extract was reconstituted in 120 μL of mobile phase and 30 μL was injected into the LC–MS/MS system.

2.4. LC–MS/MS

2.4.1. Chromatography

LC was performed using a Waters Alliance 2695 separation module (Waters, Milford, MA, US). Analytes were separated on a XBridge C_{18} column (150 mm × 2.1 mm, 3.5 μm) (Waters), eluted isocratically with methanol:0.1% formic acid (80:20, v/v), delivered at a flow rate of 0.3 mL/min. The total run time of the method was 13 min.

2.4.2. Mass spectrometry

A Quattro Ultima tandem MS (Waters) fitted with a Z-Spray ion interface was used for all analyses. Ionization was achieved using electrospray in the positive ionization mode (ESI+). The following conditions were found to be optimal for the analysis: capillary voltage, 1.0 kV; source block temperature, 120 °C, desolvation gas (nitrogen) was heated to 350 °C and delivered at a flow rate of 800 L/h. The appropriate multiple reaction monitoring (MRM) conditions for the individual analytes and their respective deuterated analogues were determined by direct infusion into the MS/MS. The cone voltage (CV) was adjusted to maximize the intensity of the protonated molecular species $[M+H]^+$ and collision-induced dissociation of each protonated molecule was performed. Collision gas (argon) pressure was maintained at 2.7×10^{-3} mbar and the collision energy (eV) adjusted to optimize the signal for the most abundant product ions, which were subsequently used for MRM analysis.

All aspects of system operation and data acquisition were controlled using MassLynx NT 4.2 software with automated data processing using the TargetLynx™ software (Waters). The statistical treatment of data was carried out using Excel 2000 (Microsoft).

2.5. LC–MS/MS assay validation

The analytical validation was performed according to the recommendations of Peters and Maurer [15,16], Shah et al. [17] and the SOFT/AAFFS Laboratory Guidelines [18].

2.5.1. Linearity, limit of quantification (LOQ), limit of detection (LOD), precision and accuracy

Quantification was performed by integration of the area under the specific MRM chromatograms in reference to the integrated area of the deuterated analogues. Freshly prepared working solutions of 200, 50, 12.5 and 2.5 μg/L for THC and 11-OH-THC, and of 800, 200, 50 and 10 μg/L for THC-COOH in methanol were used to prepare blood calibrators at a concentration of 40, 20, 15, 10, 5, 2, 1