Journal of Chromatography B 1074–1075 (2018) 1–7

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous accelerated solvent extraction and hydrolysis of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid glucuronide in meconium samples for gas chromatography–mass spectrometry analysis

Cinthia de Carvalho Mantovani[a], Jefferson Pereira e Silva[a,*], Guilherme Forster[a], Rafael Menck de Almeida[a], Edna Maria de Albuquerque Diniz[b], Mauricio Yonamine[a]

[a] Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes 580, 13B, 05508-900 São Paulo, SP, Brazil

[b] Pediatrics Department, University Hospital of São Paulo, Av. Prof. Lineu Prestes 2565, 05508-900 São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Meconium
Cannabinoids glucuronides
Accelerated solvent extraction
Hydrolysis optimization
GC–MS

ABSTRACT

Cannabis misuse during pregnancy is associated with severe impacts on the mother and baby health, such as newborn low birth weight, growth restriction, pre-term birth, neurobehavioral and developmental deficits. In most of the cases, drug abuse is omitted or denied by the mothers. Thus, toxicological analyzes using maternal-fetal matrices takes place as a suitable tool to assess drug use. Herein, meconium was the chosen matrix to evaluate cannabis exposure through identification and quantification of 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic (THCCOOH). Accelerated solvent extraction (ASE) was applied for sample preparation technique to simultaneously extract and hydrolyze conjugated THCCOOH from meconium, followed by a solid-phase extraction (SPE) procedure. The method was developed and validated for gas chromatography–mass spectrometry (GC–MS), reaching hydrolysis efficiency of 98%. Limits of detection (LOD) and quantification (LOQ) were, respectively, 5 and 10 ng/g. The range of linearity was LOQ to 500 ng/g. Inter and intra-batch coefficients of variation were < 8.4% for all concentration levels. Accuracy was in 101.7–108.9% range. Recovery was on average 60.3%. Carryover effect was not observed. The procedure was applied in six meconium samples from babies whose mothers were drug users and showed satisfactory performance to confirm fetal *cannabis* exposure.

1. Introduction

Cannabis sativa L. is the most widely cultivated, trafficked and abused illicit drug in the world [1] and its use has been present in human life since early times. The plant was used as a source of textile fiber, ingredients in traditional medicine and religious rituals [2,3].

Until now the plant continues to be widely used and according to the World Drug Report (2017), in the last decade women began to play an important role in the pattern of drug abuse, including cannabis, but there is no information about use during the gestational period [4].

In the United States [5] approximately 3.4% of pregnant women use marijuana; in Europe this percentage reaches 10% [6] while in Brazil a research involving teenagers, pointed out that 4.1% used marijuana in the third trimester of pregnancy [7].

Exposure to cannabis has been associated with some neonatal outcomes such as low birth weight, intra-uterine growth restriction, preterm birth, neurobehavioral developmental deficits [8–12] and decreased motor development through exposure by breastfeeding [13], but the cause-effect relationship remains controversial [14].

Even though the health impacts are incompletely elucidated, cannabis cannot be considered as innocuous or safe because it presents a variety of harmful agents [15,16] and can act as a stressor due to its strong psychotropic activity [17–21], classically associated to impairment of newborn health and lifelong consequences [10,22,23]. Cannabinoids can force a supra physiological stimuli on the endocannabinoid signaling system (CB1 and CB2 receptors) during fetal life resulting in altered brain circuit formation [24–27].

Therefore, it is clear that the monitoring of drug consumption should be a priority in childbearing. In this sense, the maternal report can be helpful, but it is not completely reliable [7,28]. Thus, toxicological tests are considered the safest way to assess drug exposure because can identify a biotransformation product and/or parental drug in maternal-fetal matrices [29].

To achieve this, meconium has been used as a source for assessment of prenatal exposure to all classes of illicit drugs [30–36] and other intoxicants [37–42]. The biological sample starts forming by the 12th

* Corresponding author.
E-mail address: silvajp@usp.br (J.P.e. Silva).

https://doi.org/10.1016/j.jchromb.2018.01.009
Received 6 November 2017; Received in revised form 19 December 2017; Accepted 6 January 2018
Available online 07 January 2018
1570-0232/

and 16th weeks of gestation and can accumulate xenobiotics that came directly from maternal circulation until birth, covering about the last 20 weeks of fetal exposure to drugs [43].

Especially for the detection of cannabinoids in meconium, some analytical methods were developed employing multiple steps and organic solvents [6,44–51] in an attempt to overcome the complexity of the matrix and the major challenge: the hydrolysis of glucuronide conjugates. Hydrolysis is a crucial step in sample preparation on which this method's sensitivity largely depends and is performed in both alkaline and/or enzymatic procedures. The last one can make the sample handling time-consuming and expensive [46–49].

Generally, the targeted analytes are the Δ9-tetrahydrocannabinol (THC) and its major metabolites, 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) and 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC), considered the most predominant in human biological matrices [52] and the recently published methods focus on feasibility, efficiency, simplicity and affordability [50,51].

Accelerated solvent extraction (ASE) encompasses these indispensable requisites to establish a reproducible method and meets an environmental appeal of using less solvent in faster procedures than conventional ones. ASE combines higher temperatures and pressures, altering the physicochemical properties of organic and aqueous solvents and improving the efficiency of the extraction, recovery and solubility of solutes by enhancing diffusion coefficients of mass transfer from matrix to solvent [53,54].

This technique was previously used in toxicological analysis and showed satisfactory results [36]. The aim of the current study was to develop a gas chromatography–mass spectrometry (GC–MS) method for the determination of THCCOOH in meconium samples, using the ASE to simultaneously perform the procedures of sample extraction and hydrolysis of the metabolite.

2. Material and methods

2.1. Standards and chemicals

Standards of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH), 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid glucuronide (THCCOOH-glucuronide) and the internal standard (IS) 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid-d_3 (THCCOOH-d_3) were purchased from Cerilliant (Cerilliant Corp., Round Rock, TX, USA) as 100 µg/mL sealed glass ampules (methanolic solutions). Working solutions of THCCOOH, THCCOOH-glucuronide and THCCOOH-d_3 were prepared in methanol at a concentration of 1.0 µg/mL and stored at −20 °C until analysis. Methanol, glacial acetic acid, ammonium hydroxide, sodium hydroxide, hydrochloric acid, acetonitrile, acetone, ethyl acetate and hexane were obtained from Merck (Darmstadt, Germany). Diatomaceous earth was used as an inert material to fill the ASE cell extraction, and cellulose filters were purchased from Thermo Scientific (Sunnyvale, CA, USA) and pH indicator strips (0–14) from Merck (Darmstadt, Germany). Bond Elut Certify II (130 g, 3 mL) solid phase cartridges were obtained from Agilent Technologies (Lake Forest, CA, USA). Derivatization reagent *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (MTBSTFA) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Instrumentation and GC–MS conditions

The accelerated solvent extraction procedure was performed in ASE 100 equipment from Dionex Corporation (Sunnyvale, CA, USA). Solid phase extraction was accomplished with a GX-274 ASPEC automated system, equipped with two 406 Dual Syringe and Trilution LH software, from Gilson (Middleton, WI, USA). Analysis were carried out on an Agilent Technologies 6850 gas chromatograph coupled with a 5975 mass spectrometry detector, and equipped with a G4513A autosampler (Palo Alto, CA, USA). Chromatography separation was achieved with a fused silica capillary column HP-5MS (30 m × 0.25 mm × 0.1 µm), also purchased from Agilent Technologies. The injections were made in splitless mode at 260 °C. The injection volume was 2.0 µL of the extract. High purity helium gas, with a flow rate of 1.0 mL/min was used as a carrier gas. The oven temperature was programmed at 120 °C for 2 min, increasing to 290 °C at 20 °C/min (2 min hold). The chromatographic total run time was 12.5 min. The transfer line, ion source and quadrupole temperature were, respectively, 280 °C, 230 °C and 150 °C. The electron ionization mass spectra was acquired by a selective ion monitoring (SIM) mode. The following ions were used for analyte identification (underlined ions were selected for quantification): *m/z* <u>515</u>, 557, 572 for THCCOOH and *m/z* <u>518</u>, 560 and 575 for THCCOOH-d_3. The acceptance criteria for analyte identification and quantification were: (1) retention time within ± 2% from calibrators; (2) relative ion intensity ± 20% from calibrators intensity ratios.

2.3. Meconium collection and quality control samples

Meconium samples were collected at the University Hospital of São Paulo after the mother's agreement. The samples were collected from the diapers up to 24 h after birth, and stored at −20 °C until analysis. Forty specimens with no record of cannabis use by the mother were confirmed negative by GC–MS at the limit of detection. They were pooled, mixed thoroughly and used to prepare the quality control samples, calibrators and spiked samples. Quality control samples (QC) were obtained by spiking blank meconium samples with concentrations of 30 ng/g (low control), 200 ng/g (medium control) and 400 ng/g (high control) of THCCOOH-glucuronide.

2.4. Preparation of meconium samples

Cellulose filters were placed at the bottom and at the top of a 10 mL ASE cell extraction. Afterwards, aliquots of meconium samples (500 ± 20 mg), 50 µL of IS working solution (1.0 µg/mL) and 2.4 g of diatomaceous earth were added into the ASE cell, which was further attached to the equipment. The ASE conditions were set up as follows: 5 min of heating time, 2 min of static cycle and 1 min of purge time. The procedure was accomplished with sodium hydroxide solution (0.4 mol/L), used as solvent extraction, 120 °C of temperature and 1500 psi of pressure. Approximately 10 mL of the extract was collected in 40 mL glass bottles, which was then neutralized to pH 7.0 with nearly 1.5 mL of 6 mol/L hydrochloric acid. The sample solution was transferred to glass tubes (15 mm × 150 mm) and submitted to automated solid phase extraction. A GX-274 ASPEC system was programmed to condition Bond Elut II cartridges with 2 mL of methanol and 2 mL of deionized water. The flow rate was set at 3 mL/min. After conditioning, the sample solution was loaded on the SPE cartridge at flow rate of 1.5 mL/min. The cartridge was washed with 2 mL of water/acetonitrile/ammonium hydroxide (85:15:1) at 4 mL/min flow rate and then dried under nitrogen stream at 10 psi pressure. The analytes were eluted with 3 mL hexane/ethyl acetate/acetone/glacial acetic acid (54:18:27:1). The eluate was allowed to dryness at 40 °C under nitrogen stream. The extract was derivatized with MTBSTFA according to Wiergowski [55], using 50 µL for 30 min at 70 °C and analyzed by GC–MS.

2.5. Hydrolysis optimization

The hydrolysis of THCCOOH-glucuronide, carried out simultaneously to ASE procedure, was optimized by applying a multivariate statistic technique, the response of surface methodology (RSM). The software Design-Expert® (version 8) was used to design the Box-Behnken experiment and to evaluate the statistical analysis of the surface response results. The three factors and its relating levels studied through the RSM were as follow: NaOH concentration (0.01, 0.2 and 0.4 mol/L); temperature (40, 80 and 120 °C); and static time (2, 6 and 10 min). Blank meconium samples (500 mg) were spiked with 151 µL of