Extraction of THC Metabolites in Urine using the Empore™ Membrane C18 Extraction Cartridge

Application Note

Cannabis

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Abstract

In this application note, the Empore membrane solid phase extraction cartridge is used to extract THC metabolites from urine samples as part of a routine sample preparation procedure. Specifically, the THC-OOH metabolite was quantified as part of this method.

Introduction

Currently in the cannabis market, there is a growing need for purifying extracts and also quantifying tetrahydrocannabinol (THC) and its various metabolites. Sample preparation by solid phase extraction (SPE) is one such method for achieving both purification and extraction goals. Empore™ specifically is membrane type of SPE that offers a number of advantages over traditional loose-packed SPE products.

In the Empore™ membrane, sorbent particles are trapped in a bed of an inert polymer matrix. Empore™ SPE cartridges specifically, are made with sealing ring that secures the SPE membrane to the bottom of a medical-grade polypropylene barrel. On the top of the SPE membrane, there is a 8-layer pre-filter layer, which is composed of polypropylene microfiber layers with different pore sizes. The design of Empore™ SPE cartridges eliminates channeling effects, and there is no shedding of fine particulates, which is commonly observed with traditional loose-packed SPE. In this experiment, a 3 mL C18 (4215SD) membrane solid-phase extraction cartridge from Empore™ was used to extract the THC metabolite 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in urine. THC-COOH was quantified by GC-MS analysis [1,2].

Experiment Setup

1.1 Instruments and Reagents

THC-COOH (100.0±0.6 µg/mL) and MSTFA + 1% TMCS silylation reagent were both purchased from Sigma-Aldrich. Artificial urine (pH: 5.7-500mL) was purchased from Fuzhou Feijing Biotechnology Co., Ltd. (Fuzhou, China). Ethyl acetate (chromatographically pure), methanol (chromatographically pure), and n-hexane (chromatographically pure) were all purchased from Thermo Fisher Scientific. All reagents are of analytical grade or higher. Extraction was performed using the Empore™ Membrane Solid Phase Extraction cartridge: 3mL C18, CDS Analytical (model No. 4215SD; Fisher Sci. PN 13-110-013; VWR PN 76333-122; Millipore-Sigma PN 66872-U). Analysis was done using a 7890B-5977B Agilent GC-MS instrument, Agilent.

1.2 Working and calibration solutions

THC-COOH standard working solution was prepared in methanol at 100 ng/mL. THC-COOH-d3 internal standard solution was prepared in methanol at 1,000 ng/mL. Calibration solution: Different volumes of THC-COOH working solution were added to 6, 20 mL vials such that the THC-COOH mass was 3 ng, 15 ng, 30 ng, 60 ng, and 300 ng. After adding 50 µL of internal standard solution to the vials, the solutions were dried with a gentle stream of nitrogen at 40 °C, and then 100 µL of derivatization reagent (MSTFA+1% TMCS) was added to each vial.

Derivatization was formed at 60 °C for 20 min. Each of the solution was transferred to a GC vials and diluted to 1.0 mL with n-hexane for GC-MS.

1.3 Experiments for recovery

1.3.1 Sample Preparation

16 x 3 mL urine samples were each placed in 20-mL sample tubes. 200 µL THC-COOH standard solution (spiking amount is 20 ng) was added to 6 of them, and 15 ng, 30 ng, 60 ng, 150 ng, and 300 ng were added to the other 10 urine samples with two duplicates for each. 300 µL of potassium hydroxide solution (10 M) was added to each sample, hydrolyzed in a water bath at 60 °C for 15 min, removed, and cooled to room temperature. The pH was then adjusted to 4.0-4.5 with glacial acetic acid.

1.3.2 Extraction and concentration

The Empore™ C18 (4215SD) cartridges were pre-activated with 150 µL methanol and then loaded with the solutions from section 1.3.1 of the Experimental Setup (sample was allowed to flow down by gravity). After loading, the extraction cartridges were rinsed with 750 µL methanol-water solution (50:50), dried under vacuum for 5 min, and finally eluted with 800 µL n-hexane:ethyl acetate solution (75:25 v:v). The eluates were concentrated/ derivatized with the same procedure as described in section 1.2 for standard solutions.

1.3.3 GC-MS method:

The injection port was run in splitless mode at a temperature of 290 °C. The He carrier gas flow rate was 1.0 mL/min. The column temperature program used for this work is as follows: hold at 100 °C for 1 min, then increase to 300 °C at a rate of 30 °C/min and hold for 5 min. The analyte quantification ions are shown in Table 1.

Results

The THC-COOH total ion chromatogram is shown in Figure 1. The linearity of the 5-level calibration curve is R^2 = 0.9998. The recovery results of THC-COOH spiked in urine at the level of 20 ng are listed in Table 2. The recovery results of THC-COOH spiked in urine at the level of 15 ng, 30 ng, 60 ng, 150 ng, and 300 ng are listed in Table 3.

Table 1. Qualitative and quantitative ions of THC-COOH and THC-COOH-d3

#	Compound	Qualitative and quantitative ions	Retention time (min)
1	THC-COOH-d3	374/476/491	12.09

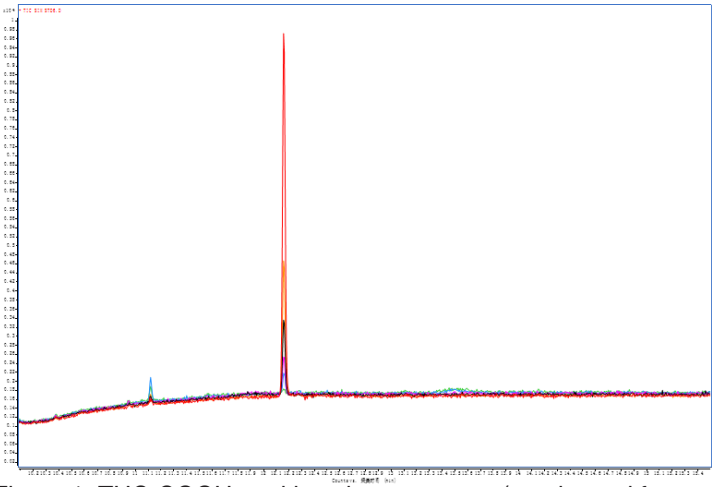

Figure 1. THC-COOH total ion chromatogram (overlapped for different levels of standards, ions of THC-COOH only)

Conclusions

The experiment demonstrated a simple and effective method for enrichment and extraction of tetrahydrocannabinol metabolites THC-COOH in urine using the Empore™ 4215SD solid-phase extraction cartridges. In the experiment, the THC-COOH-spiked urine samples were subjected to pretreatment steps such as hydrolysis, solid-phase extraction purification, concentration, and derivatization. Spiked recoveries rates of THC-COOH were obtained by GC-MS analysis. All recoveries were >83%, which is satisfactory for routine analysis. The experiment shows that Empore™ 4215SD solid-phase extraction cartridge is suitable for the extraction and purification of THC-COOH in urine.

References

[1] Marilyn, et al. “Blood Cannabinoids. I. Absorption of THC and Formation of 11-OH-THC and THCCOOH During and After Smoking Marijuana*.” Journal of Analytical Toxicology (1992).

[2] Giroud, C., et al. “Delta(9)-THC, 11-OH-Delta(9)-THC and Delta(9)-THCCOOH plasma or serum to whole blood concentrations distribution ratios in blood samples taken from living and dead people.” forensic science international 123.2-3(2001):159.

Table 2. Spike recovery results for the level of 20 ng

Spike Level (ng)	Recovery (%)							
	1	2	3	4	5	6	Average (%)	RSD (%)
20	90.2	94.5	89.9	87.4	88.5	83.7	89.0	4.0

Table 3 Spike recovery results for the level of 15 ng, 30 ng, 60 ng, 150 ng, and 300 ng

#	Spike level (ng)	Recovery (%)		Average (%)
		1	2	
1	15	82.2	96.4	89.3
2	30	101.8	109.4	105.6
3	60	89.2	78.7	83.9
4	150	93.1	101.3	97.2
5	300	91.4	110.1	100.7