Solid Phase Extraction and Analysis of THC and Carboxy-THC from Whole Blood using a Novel Fluorinated SPE Sorbent and Fast LC-MS/MS

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ABSTRACT

In this study, solid phase extraction (SPE) is described using a novel fluorinated (Heptadecafluorotetrahydrodecyl (C10H4F17)) phase to isolate THC and its primary metabolite Carboxy-THC from whole blood samples. SPE was performed in hydrophobic mode after samples of whole blood were precipitated with acetonitrile. After applying the sample to the SPE column in aqueous phosphate buffer (pH 7) the sorbent was washed with deionized water, phosphate buffer (pH 7), and dried. The SPE column was eluted with a solvent consisting of ethyl acetate/ hexanes (50:50) containing 2% acetic acid. The eluate was collected, evaporated to dryness and dissolved in mobile phase (50 µL) for analysis by fast LC-MS/MS in positive/ negative MRM mode. Chromatography was performed in gradient mode employing a C18 column and a mobile phase consisting of acetontitrile (containing 0.1% formic acid) and 0.1% aqueous formic acid. The total run time for each analysis was less than 5 minutes.
The limits of detection/ quantification for this method were determined to be 0.1 ng/ mL and 0.25 ng/ mL, respectively. The method was found to be linear from 0.25 ng/ mL to 50 ng/ mL (r2≥0.995). Recoveries of the individual cannabinoids were found to be greater than 85%. In this report, results of authentic samples analyzed for THC and Carboxy-THC are reported using this new methodology.

INTRODUCTION

Cannabis (marijuana) is one of the most widespread and frequently used drugs in the world. It is generally smoked as a cigarette or in a pipe or water pipe ("bong"). The active ingredient, Δ9-THC, induces feelings of euphoria and relaxation, but also impairs perception and cognizance. Although many arguments have been made for the legalization of marijuana, it remains an illicit drug in most countries. Because of its widespread use, it is often necessary to analyze biological samples, usually urine or blood, for cannabinoid use. Use of these drugs is confirmed by detection and/ or quantitation of THC and its major metabolite, 11-nor-9-carboxy- Δ9-tetrahydrocannabinol ((THC-COOH) Carboxy-THC)

Currently, screening for these compounds is often accomplished by immunoassay followed by GC/MS for confirmation and/or quantitation.. However, due the availability of LC/MS/MS , many frequently used procedures including THC are being developed away from the traditional GC-MS onto this instrument. LC/MS/MS is a complementary technique to GC/MS and the very compounds that are challenging to analyze by GC/MS, i.e. polar, amines, and semi-volatile compounds, are ideal candidates for LC/MS/MS analysis. Furthermore, sample preparation is often greatly simplified as the final step of sample preparation i.e. "derivatization" can be omitted. With the addition of fast LC, each run can be performed in under 5 minutes.

MATERIALS AND METHODS

Reagents

THC and Carboxy-THC and their deudurated analogs, were purchased from Cerilliant. Formic acid (99%) was from Acros Organics, all other solvents were HPLC grade or better and were obtained from Fisher Scientific.

A standard stock reference solution of THC and Carboxy-THC(1.0 µg/ml) was prepared in acetonitrile. THC and Carboxy-THC standards were prepared by spiking drug free blood at the following concentrations: 0.25, 1.0, 2.0, 5.0, 10 and 50 ng/mL. Another stock reference solution was prepared in acetonitrile (1.0 µg/ml) and was used for the preparation of controls at 4.0 and 15.0 mg/L. THC and Carboxy-THC concentrations in the controls blood were calculated from linear regression of the standard responses based on the peak-area ratio.

Extraction

In this procedure, after the addition of the internal standards THC-D3 and Carboxy-THC-D3) to 1 ml of whole blood, 2 mL of ice cold acetonitrile were added dropwise whilst mixing. The samples were allowed to stand for 10 minutes, after which the samples were centrifuged (10 minutes at 3000 rpm). Each supernatant was decanted into a clean tube and evaporated to about 200 µl. Then to each tube was added 5 mL of pH 7 phosphate buffer (0.1 M) prior to solid phase extraction. The SPE columns (Fluoro-C10 (UCT Inc.,)) (6mL, 200 mg) were conditioned with methanol, pH 7 buffer (3, 3, mL, respectively) after which, the samples were loaded. The SPE columns were washed with 3 mL DI water, dried, and washed again with 3 mL hexanes then dried again for 5 minutes under full vacuum. Following elution of THC/Carboxy-THC with 2 mL of hexane: ethyl acetate (1/1) with 2% acetic acid, the eluents were collected and evaporated to dryness. The residue was reconstituted with 50 µl of the methanol solution prior to the chromatographic analysis.

Instrumentation

Liquid chromatography equipment consisted with a Shimadzu Prominence (two pumps LC-20AD, autosampler SIL-20AC, and column oven CTO-20AC), was performed using Imtakt™ C_{18} column (50x 2.1mm, 5 µm), at 0.55mL/min. flow using a gradient program. The mobile phase program: (A) 0.1% aqueous formic acid) / (B) acetonitrile containing 0.1% formic acid was started at 50% (B) for 0.5 min, increasing to 90% (B) over 1.5 minute, and holding at 90% B for one minute before returning to 50% (B) and equilibrated for 2 minutes. The total chromatographic run time for each analysis was 4.5 minutes including equilibration time.

MS/MS analysis was conducted using an Applied Biosystems 3200Q Trap instrument equipped with ESI in negative ion mode for Carboxy-THC and D3 and was operated with multiple reaction monitoring (MRM) under the following conditions: curtain gas 15, collision gas medium, ion spray voltage -4500V, temperature 650 °C, ion source gas(1) 50, ion source gas (2) 50. The following transitions were monitored (quantification ions underlined): m/z 343.1 → 299.3 and 245.3 for Carboxy-THC, and m/z 346.1 → 302.3 and 248.3 for Carboxy-THC-D3. Positive ion mode was employed for THC/ THC-D3 under the following conditions: curtain gas 15, collision gas medium, ion spray voltage 5000V, temperature 650 °C, ion source gas(1) 50, ion source gas (2) 50. The following transitions were monitored (quantification ions underlined): m/z 315.2 → 193.2 and 123.1 for THC, and m/z 318.2 → 196.2 and 123.1 for D3-THC.

RESULTS AND DISCUSSION

Linearity (r2 >0.995) was achieved from 0.25 ng/mL to 50 ng/mL, (THC/ Carboxy-THC) and the limits of detection was determined to be 0.1 ng/mL for both THC and Carboxy-THC respectively. The limits of quantification was 0.25 ng/mL for both THC and Carboxy-THC, respectively. Recoveries were > 85% for THC and > 89% for Carboxy-THC, respectively measured over the calibration range. Intra and inter-day precision was less than 4% and 6%, respectively for THC and less than 6% and 8%, respectively for Carboxy-THC Ion suppression studies revealed that suppression of monitored ions was less than 6%.

Fig. 1 Graph of Carboxy-THC extracted from whole blood over the calibration range (0.25 to 50 ng/ mL)

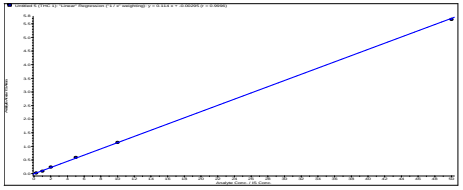


Fig. 2 Graph of THC extracted from whole blood over the calibration range (0.25 to 50 ng/mL)

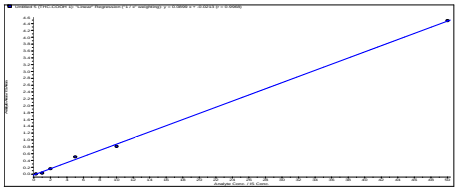


Fig. 3 Chromatogram of THC/ Carboxy-THC (and IS) at LOD 0.1 ng in whole blood (1 mL) Rt: THC: 2.57 minutes, THC-D3: 2.55 minutes Carboxy-THC: 2.07, Carboxy-THC-D3: 2.06 minutes

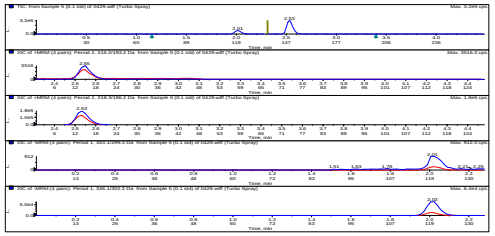
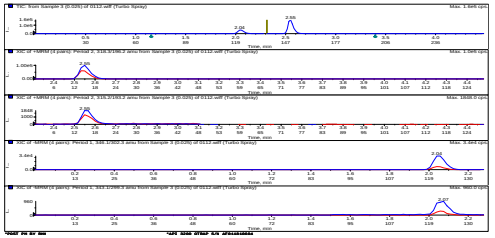


Fig. 4 Chromatogram of THC/ Carboxy-THC (and IS) spiked into whole blood at LOQ (0.25 ng/ mL) Rt: THC: 2.57 minutes, THC-D3: 2.55 minutes Carboxy-THC: 2.07, Carboxy-THC-D3: 2.06 minutes



CONCLUSIONS

This poster describes a procedure, where a novel fluorinated SPE sorbent extraction methodology coupled to fast LC-MS/MS, a highly efficient technique has been developed. The use of this process should greatly assist analysts in the field of drug related driving cases quickly resolve the issues involved in reporting values for both THC and its primary metabolite (Carboxy-THC) in whole blood samples. Comparison of re-analyzed cases with their previous historical data has shown the effectiveness of this new, improved methodology.