K2 Rapid Quantification of THC and Carboxy–THC in Blood by LC–MS/MS

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After attending this presentation, attendees will understand a simple and improved solid phase extraction (SPE) method for analyzing THC and THC-COOH in whole blood.

This presentation will impact the forensic community by assisting forensic toxicologists/analysts in implementing a simple solid phase extraction procedure coupled with LC-MS/MS for low level quantification of THC and THC-COOH in whole blood samples.

In this procedure, after the addition of the internal standards (D3- THC and D3-THC-COOH) to 1 ml of whole blood, 2 mL of ice cold acetonitrile were added dropwise while 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 mixed with 5 mL of pH 7 phosphate buffer (0.1 M) prior to solid phase extraction. The mixed mode SPE columns (C_8/SAX) 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 / THC-COOH with 2 mL of hexane: ethyl acetate (1/1), the eluents were collected and evaporated to dryness. The residue was reconstituted with 100 µl of the mobile phase solution.

Liquid chromatography was performed using 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 a tandem mass spectrometer equipped with ESI in negative ion mode for THC-COOH/ D3-THC-COOH 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 THC-COOH, and m/z 346.1 → 302.3 and 248.3 for D3-THC- COOH. Positive ion mode was employed for THC/ D3-THC 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.

Linearity (r^2 >0.99) was achieved from 0.25 ng/mL to 50 ng/mL, (THC/ THC-COOH) and the limits of detection were determined to be 0.1 ng/mL for THC and 0.25 ng/mL for THC-COOH, respectively. The limits of quantification were 0.25 ng/mL for THC and 0.5 ng/mL for THC-COOH, respectively. Recoveries were > 92% for THC and > 87% for THC-COOH, respectively measured at a target value of 4.0 ng/mL. Intra and inter-day precision was less than 7% and 11%, respectively for THC and less than 8% and 12%, respectively for THC-COOH. Ion suppression studies revealed that suppression of monitored ions was less than 6%.

This SPE method coupled with and fast LC-MS/MS provides a simple, sensitive, and reproducible quantitative method for the analysis of THC and its primary metabolite in whole blood. This procedure should be of great assistance to those analysts actively involved with the LC-MS/MS analysis of these drugs in biological matrices.

THC and Metabolite, Solid Phase Extraction, LC-MS/MS

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