K26 Optimizing the Best pH (3 or 7) for the Extraction of Cannabinoids From Whole Blood Samples in Drugs and Driving Cases

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After attending this presentation, attendees will be able to choose the most efficient pH value for extracting cannabinoids (tetrahydrocannabinol (THC), 11-hydroxytetrahydrocannabinol (THC-OH), tetrahydrocannabinol-carboxylic acid (THCA), cannabidiol (CBD), and cannabinol (CBN)) from whole blood samples employing available Solid Phase Extraction (SPE) cartridges and liquid chromatography-tandem mass spectrometry.

This presentation will impact the forensic science community by offering analysts operating in forensic facilities information regarding the extraction and analysis of popular cannabinoids encountered in drugs and driving casework using solid phase extraction and liquid chromatography-tandem mass spectrometry.

Method: Samples of whole blood ((1mL) calibrators, controls, and case samples) were spiked with THC, THC-OH, THCA,CBD, and CBN (plus THC-d3, THC-OH-d3, THCA-d3, and CBD-d3). Samples were precipitated with 2mL of acetonitrile. The acetonitrile was removed and evaporated to 500µL, then diluted with either A (5mL of 0.1M aqueous hydrochloric (pH 3)) or B (5mL of 0.1M phosphate buffer (pH 7)). This comparison study was performed using validation guidelines at Massachusetts State Police Crime Laboratory.

Extraction of the A samples was performed on commercially available SPE columns (C8/Aminopropyl). These SPE columns were conditioned with methanol, deionized (DI) water, and 0.1M aqueous hydrochloric acid (3mL, 3mL, 1mL, respectively) and the samples loaded onto the SPE cartridges at 1mL/ minute. The SPE columns were washed with DI water and 30/70 acetonitrile-0.1M HCl (3mL) before drying (10 minutes), after which the cannabinoids (and internal standards) were eluted with 3mL of a hexane/ethyl acetate/acetic acid (49:49:2) mixture. The eluates were evaporated to dryness under nitrogen and dissolved 100µL of methanol for analysis. The B samples were extracted on the same type of SPE columns pre-conditioned with methanol, DI water, and 0.1M phosphate buffer (pH 7) (3mL, 3mL, 1mL, respectively) after which the pH 7 buffered samples were loaded onto the SPE columns at 1mL/ minute. The SPE columns were washed with pH 7 phosphate buffer (3mL) before drying. The cannabinoids were eluted with 3mL of the same elution solvent. The eluates were evaporated to dryness under nitrogen before being dissolved in 100µL of methanol for analysis.

Liquid chromatography was performed in gradient mode employing a 50mm x 2.1mm (5µm) C_{18} analytical column and a mobile phase consisting of acetonitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 90% acetonitrile in 4.0 minutes and then back to 5% for re-injection. The total run time for each analysis was less than 5 minutes.

Tandem mass spectrometry was performed in positive/negative Multiple Reaction Monitoring (MRM) modes. The following transitions were monitored (quantification transition ions underlined): THC m/z: 315.3 to 193.2, 123.2; THC-d3: m/z 318.3 to 196.2, 123.1; THC-OH: m/z 331.1 to 193.1, 201.1; THC-OH-d3: m/z 334.3 to 196.2, 133.1; THCA: m/z 343.3 to 299.1, 245.1; THCA-d3: m/z 346.1 to 302.1, 245.1; CBD: m/z 313.2 to 245.2, 245.2; CBD-d3 m/z: 316.2 to 247.8, 182.1; CBN: m/z 311.3 to 223.2, 178.1, respectively. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of THC and related cannabinoids from 20 (completed) drugs and driving cases.

Results: The limits of detection/quantification for the SPE method were determined to be 0.5 ng/mL and 1.0ng/mL, respectively for all the cannabinoids. Both pH values (pH 3 and pH 7) methods were found to be linear from 1ng/mL to 100ng/mL (r^2>0.999). Data is presented to show that recoveries of all the cannabinoids were found to be greater than 90%. Interday and intraday analysis were found to <7% and <10%, respectively. Matrix effects were determined to be <5% for both types of extraction.

Conclusion: Extraction of cannabinoids at pH 3 demonstrates the efficient use of hydrophobic interactions, while extraction at pH 7 demonstrates the use of both hydrophobic and anion exchange interactions leading to better sensitivity and selectivity in the retention of the cannabinoids on mixed mode SPE columns leading to better evaluation of results. The procedure of extracting cannabinoids from whole blood samples using SPE at pH 7 offers better recovery values (>95%) than pH 3 (>90%). This information should assist analytical toxicologists when choosing an efficient method for the analysis of popular cannabinoids in whole blood samples.

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