PICKERING LABORATORIES

CATALYST FOR SUCCESS

ANALYSIS OF CANNABINOIDS USING HPLC WITH POST-COLUMN DERIVATIZATION

Cannabinoids are a class of terpenophenolic compounds that are associated with the pharmacological activity of cannabis. Broader acceptance of medical cannabis use increases the need for analytical methods capable of determining the active compounds of cannabis. Cannabinoids exist in the plant mainly as carboxylic acids and are converted to neutral analogs by light and heat while in storage or during the preparation of edible products. Acids are also converted to neutral analogs during GC analysis, which often causes differences in results when comparing with HPLC methods.

A new HPLC method with post-column derivatization was developed to analyze cannabinoids in cannabis plants as well as in cannabis containing edible products. This post-column method is based on reaction with Fast Blue Salt reagent under basic conditions, a well-known colorforming reaction that is used in drug tests to detect cannabinoids via test-tube methods and thin-layer chromatography. Detection at 475 nm is performed using a UV/Vis detector.

Our method implements a simple extraction with acidified water/acetonitrile followed by QuEChERS sample clean-up. The same procedure is applicable to both plant materials and edible products containing cannabis. The method is suitable for analysis of the major neutral cannabinoids such as THC, CBD, CBN and CBG as well cannabinoid acids THCA-A and CBDA with high sensitivity and selectivity of detection.

METHOD

Analytical Conditions

Column: C_{18} reversed-phase column, 4.6 x 150 mm

Column Temperature: 45 ºC

Flow Rate: 1 mL/min

Mobile Phase: 70% acetonitrile – 30% sodium phosphate buffer (6 mM) pH 3.5

Injection Volume: 20 uL

Post-column Conditions

Post-column System: Pinnacle PCX or Vector PCX

Heated Reactor Volume: 1.4 mL

Temperature: 30 ºC

Ambient Reactor: 0.1 mL

Reagent 1: Dissolve 0.1 g of Fast Blue Salt in 240 mL of DI water. Add 40 mL of 1 N HCl and 720 mL of Acetonitrile. Protect the reagent from light. Use within 3 days.

Reagent 2: Dissolve 8 g of NaOH in 1L of DI water

Reagents Flow Rate: 0.25 mL/min

Detection: UV/VIS 475 nm

To avoid precipitation of aging reagents flush Pinnacle PCX regularly with 49 : 49 : 2 – water : Methanol : 0.1N HCl

Supplies for Sample Preparation

Extraction solution: Acetonitrile containing 1% of Acetic Acid / Water (50:50)

Q-SEP QuEChERS extraction salts: Restek, Cat # 26238 (AOAC 2007.01 Method)

Sample Extraction and Cleanup

Use 0.1 – 0.2 g sample size to analyze plant material and 0.2 – 0.5 g sample size to analyze edible products. Place homogenized sample into 50 mL centrifuge tube and add 30 mL of Extraction Solution. Blend using a hand held blender for 1 min. For candies, chews and other products hard or viscous products let the solution sit for 30 min before blending. Shake blended extracts for 30 min using a mechanical shaker.

Centrifuge the samples for 10 min at 4,000 rpm, decant and save the extract. Repeat the extraction with fresh 30 mL of extraction solution. Centrifuge, decant the extract and combine it with the first portion.

Place 30 mL of combined extract in 50 mL centrifuge tube. Add Q-Sep QuEChERS extraction salts to each centrifuge tube according to the manufacture instructions. Vigorously shake for 1 min. Centrifuge the sample for 10 min at 4,000 rpm. Use upper layer for analysis. Filter through 0.45 Nylon filter before injecting. If needed, dilute the sample with Methanol to fit the calibration curve.

Extraction Efficiency Study

Due to availability and price constraints of cannabinoids standards, spike-recoveries studies are not practical to evaluate extraction efficiency. We performed three re-extractions of each sample to validate the extraction procedure. It was found that extraction with two 30-mL portions of extraction solution is sufficient to extract more that 97.5% of cannabinoids. Due to relatively high levels of cannabinoids in the samples and high sensitivity of the method, additional dilution of the extract due to repeated extraction didn't negatively affect the analysis.

Calibration

The following cannabinoids were analyzed: delta-9 tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), delta-9 tetrahydrocannabinolic acid A (THCA-A) and cannabidiolic acid (CBDA).

The calibrators were prepared by diluting commercially available cannabinoids standards with methanol. The following calibration ranges were used: 1 ppm to 75 ppm for CBG, CBD, CBN, THC and CBDA; 5 ppm to 75 ppm for THCA-A and CBC. Correlation coefficient R^2 for all calibration curves exceeded 0.999 value.

Analysis of Cannabinoids in Cannabis and Cannabis-Containing Edible Products

To demonstrate method capabilities, the method was applied for analysis of cannabinoids in medical cannabis products: dry cannabis inflorescence, commercially available pre-rolls, medical cannabis chocolate chip cookies and medical cannabis sugar-free chews.

As expected, cannabis inflorescences and pre-rolls made with dry plant material contained high levels of cannabinoid acid (THCA-A). Dry plant material may also contain cannabidiolic acid (CBDA) but this compound has not been detected in any of the samples we analyzed.

TABLE 1. ANALYSIS OF CANNABINOIDS IN PLANT MATERIAL AND EDIBLE PRODUCTS

Sample	CBG	CBD	CBN	THC	THCA-A	CBC	CBDA
Dry Cannabis Inflorescence	3.76 mg/g	ND	3.15 mg/g	3.11 mg/g	142.52 mg/g	ND	ND
Pre-rolls	3.65 mg/g	ND	3.16 mg/g	28.72 mg/g	60.33 mg/g	ND	ND
Chocolate Chip Cookie	0.45 mg/g	0.51 mg/g	0.32 mg/g	3.48 mg/g	ND	0.39 mg/g	ND
Chews	0.91 mg/g	2.73 mg/g	0.74 mg/g	29.43 mg/g	1.98 mg/g	0.62 mg/g	ND

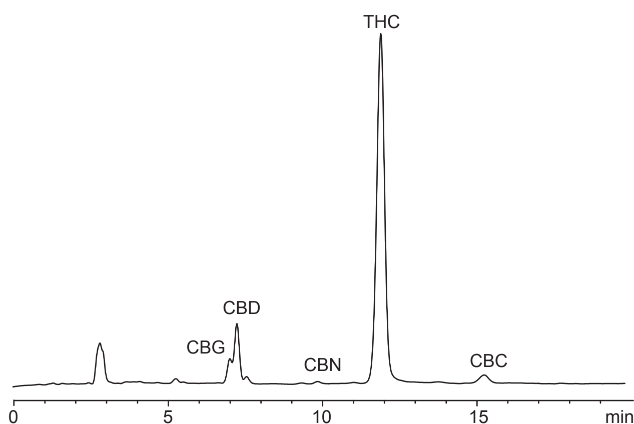

Fig 1. Chromatogram of cannabis-containing chocolate chip cookie sample

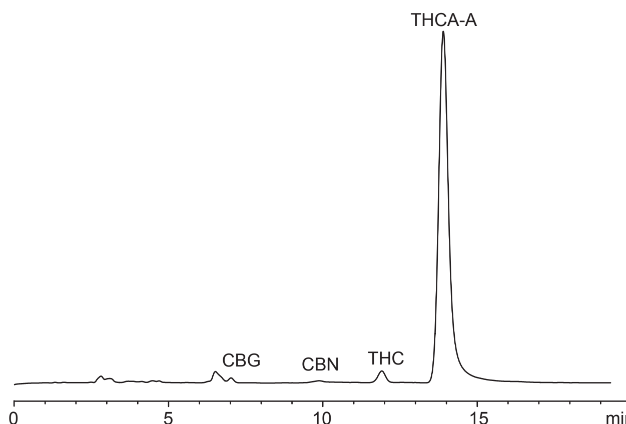

Fig 2. Chromatogram of cannabis inflorescence

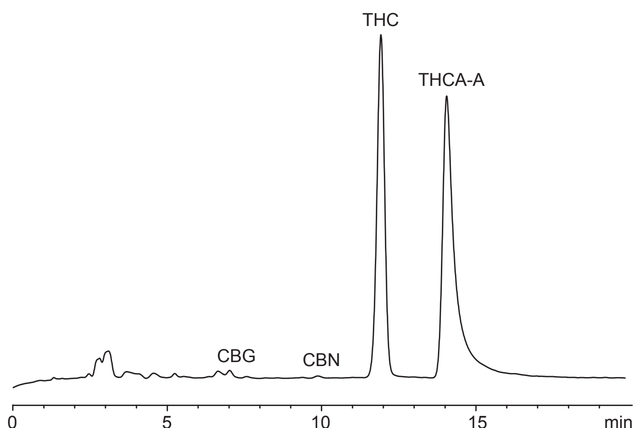

Fig 3. Chromatogram of commercially available pre-roll sample

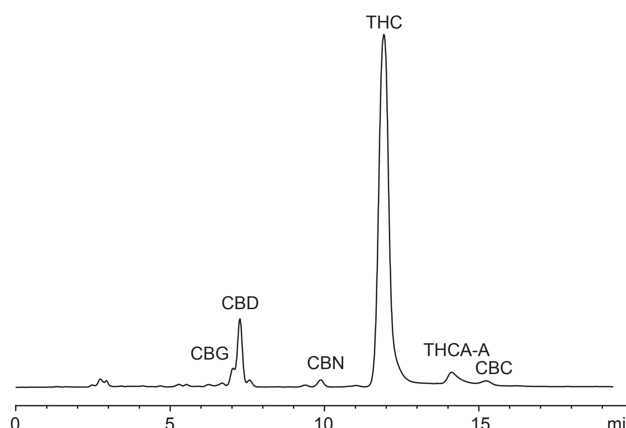

Fig 4. Chromatogram of cannabis-containing chews

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