APPLICATION NOTE

Liquid Chromatography

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# Cannabinoid Monitoring in a Variety of Edibles by HPLC-PDA

## Introduction

Current trends for the analysis of the cannabinoid content in commercially available food products point towards liquid chromatography for ensuring label-claim accuracy in product content descriptions. This analysis can be challenging, since the fortification of cannabinoid compounds has been applied to a diverse spectrum of matrices, including high sugar, high fat materials, which can make sample preparation particularly demanding.

This application describes the sample preparation and analytical method for the chromatographic separation and quantitative monitoring of twelve primary cannabinoids in the extracts of several food matrices by HPLC, using photodiode array (PDA) detection. The structures for these cannabinoids are shown in Figure 1.

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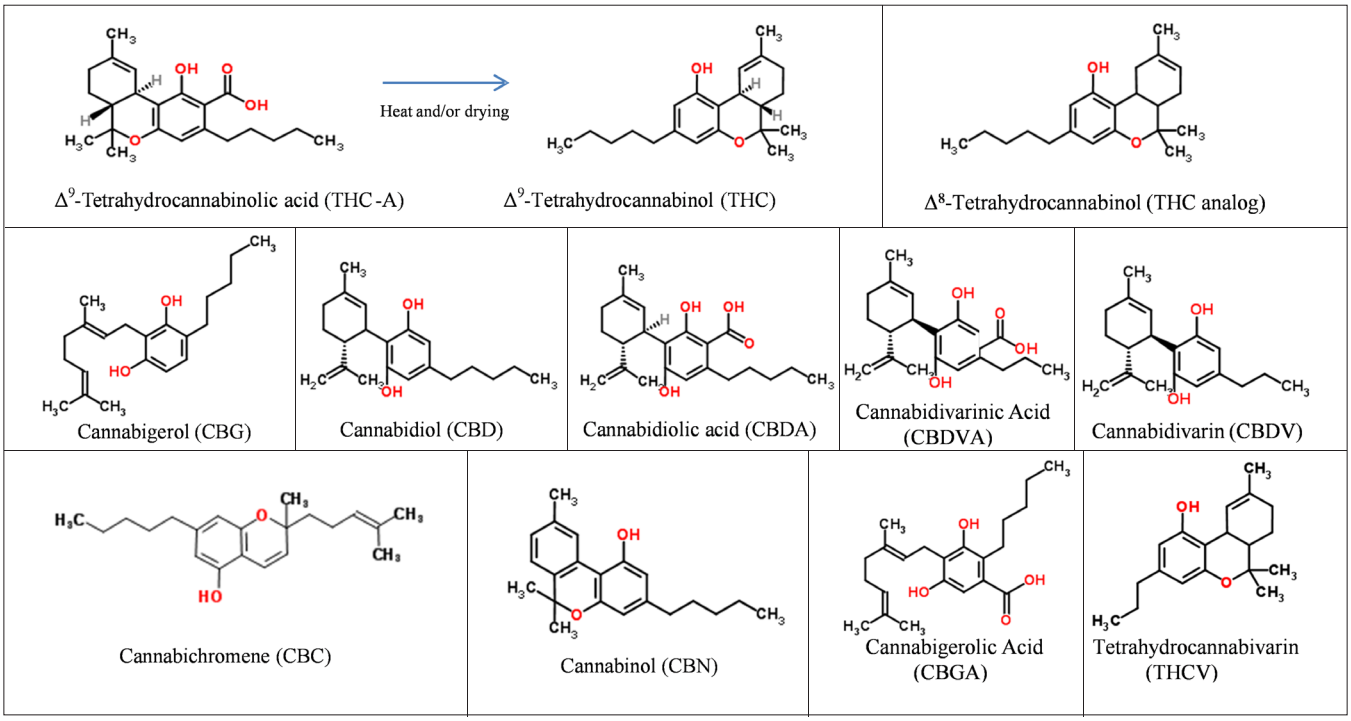


*Figure 1.* Chemical structure of the twelve cannabinoids analyzed in this study.

## Experimental

### Hardware/Software

A PerkinElmer Flexar™ HPLC system was used, including a quaternary pump, autosampler with Peltier cooling, column heater and PDA (photodiode array) detector, with 10-mm flow cell. A PerkinElmer Brownlee™ SPP C18, 2.7 µm, 3.0 x 150mm column was used for all analyses (PerkinElmer, Shelton, CT, USA). All instrument control, data analysis/processing was performed via the PerkinElmer Chromera™ CDS software.

### Method Parameters

The LC Method Parameters are shown in Table 1.

### Solvents, Standards and Samples

All solvents and diluents used were HPLC grade and filtered via 0.45-µm filters. All standard and sample extract dilutions were prepared using 80:20 methanol/water.

A 12-cannabinoid standard mix was prepared in methanol. This standard mix contained 83.3 µg/mL each of Δ9-tetrahydro-cannabinol (d9-THC), Δ9-tetrahydrocannabinolic acid (THC-A), Δ8-tetrahydrocannabinol (d8-THC), tetrahydrocannabivarin (THCV), Cannabidivarin (CBDV), Cannabidivarinic acid (CBDVA), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabinol (CBN) and cannabichromene (CBC). For calibrants, the mix was serially diluted to concentration levels of 20.8, 10.4, 5.2, 2.6, 1.3, 0.65 and 0.33 µg/mL (ppm).

*Table 1.* HPLC Method Parameters.

| | |
|---|---|
| **Column** | PerkinElmer Brownlee SPP C18, 2.7 µm, 3.0 x 150 mm (Part# N9308411) |
| **Mobile Phase** | **Solvent A:** Water with 0.1% formic acid<br>**Solvent B:** Acetonitrile with 0.1% formic acid<br>Solvent Program: |

| Step | Step Time (min.) | Flow Rate (mL/min.) | %A | %B |
|---|---|---|---|---|
| 0 (Equil) | 4.5 | 1.0 | 30.0 | 70.0 |
| 1 | 4.0 | 1.0 | 5.0 | 95.0 |
| 2 | 2.0 | 1.0 | 5.0 | 95.0 |

| | |
|---|---|
| **Analysis Time** | 6.0 min.; equilibration time: 4.5 min. |
| **Flow Rate** | 1.0 mL/min. |
| **Pressure** | 4600 psi/317 bar maximum |
| **Oven Temp.** | 40 °C |
| **PDA Detection** | Wavelength: 228 nm |
| **Injection Volume** | 10 µL |
| **Sampling (Data) Rate** | 10 pts./sec |
| **Diluent:** | 80:20 methanol/water |

Ten edible samples were initially prepared using the procedures shown in Table 2. Thereupon, the resulting sample extracts were further diluted, as also shown in Table 2. Individual extract procedures and dilutions varied, depending on the type of food matrix and expected cannabinoid content in each sample. It should be noted that all pre-analytical extraction methods were developed by the testing laboratory with considerable input from the formulation client. All analyte recovery expectations by the client were met, as confirmed by the testing laboratory.

*Table 2.* Sample list and extraction/dilution procedures for each sample.

| Sample | Weighed Amount (g) | Extractant Solvent | 4x Extract Dilution | Initial Extract Dilution | Additional Dilution | Overall Sample Dilution |
|---|---|---|---|---|---|---|
| **Cookie** | 5.0343 | 40 mL MeOH * | 300 µL Extract + 900 µL 15% $H_2O$ in ACN | 32-fold | 2-fold | 64-fold |
| **Chocolate Bar** | 5.0684 | 40 mL MeOH ** | 300 µL Extract + 900 µL 15% $H_2O$ in ACN | 32-fold | 40-fold | 1280-fold |
| **Brownie** | 5.0669 | 40 mL MeOH * | 300 µL Extract + 900 µL 15% $H_2O$ in ACN | 32-fold | 2-fold | 64-fold |
| **Rice Crispy Treat** | 5.0581 | 40 mL MeOH * | 300 µL Extract + 900 µL 15% $H_2O$ in ACN | 32-fold | 2-fold | 64-fold |
| **Gummy1** | 9.5815 | 40 mL MeOH *** | 300 µL Extract + 900 µL ACN | 16-fold | 4-fold | 64-fold |
| **Gummy2** | 0.9935 | 10 mL DMSO | 300 µL Extract + 900 µL ACN | 40-fold | 4-fold | 160-fold |
| **Hard Candy** | 1.0132 | 10 mL DMSO | 300 µL Extract + 900 µL ACN | 40-fold | 4-fold | 160-fold |
| **Lip Balm** | 1.0394 | 10 mL MeOH ** | 300 µL Extract + 900 µL 15% $H_2O$ in ACN | 40-fold | 4-fold | 160-fold |
| **Cherry Elixir** | 1.0383 | 10 mL MeOH | 300 µL Extract + 900 µL ACN | 40-fold | 2-fold | 80-fold |
| **Sour Spray** | 1.0327 | 10 mL MeOH | 300 µL Extract + 900 µL ACN | 40-fold | 2-fold | 80-fold |

* Liquid extraction from solid homogenate
** Sample first heated (melted)/sonicated and then extracted
*** Cannabinoids were only coated on surface; therefore, the whole gummy was used for extraction

All calibrants and prepared samples were subsequently filtered through 0.45-µm filters and then injected on column. The results reflect the averaged triplicate injections for all calibrants and samples.

## Results and Discussion

Figure 2 shows the chromatogram of the L6 standard mix (10.4-µg/mL) containing the twelve cannabinoids, all well resolved in under five minutes.

Per Figure 3, chromatographic repeatability was found to be exceptional, here shown via the chromatographic overlay of ten replicate 10-µL injections of the L7 standard (20.8-µg/mL).

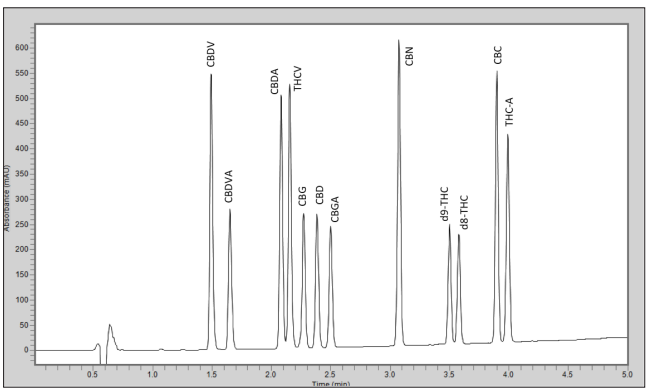


*Figure 2.* LC chromatogram showing the separation of the twelve cannabinoids in the L6 standard; λ = 228 nm.

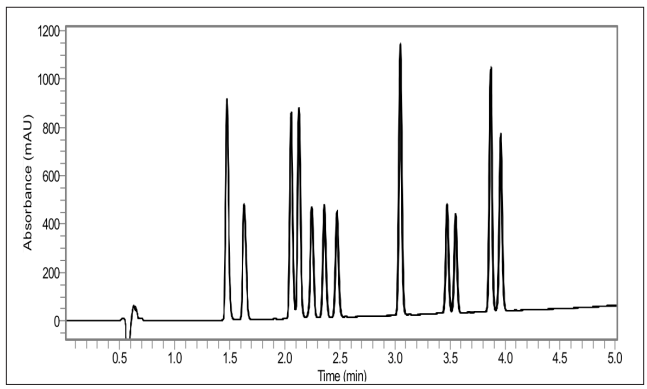


*Figure 3.* Overlay of ten replicates of the L7 standard.

Seven-level calibration fits were determined for all twelve cannabinoids. Representative linearity plots for CBDV, THC and THC-A are shown in Figure 4. The $R^2$ values for all twelve cannabinoids were above 0.999.

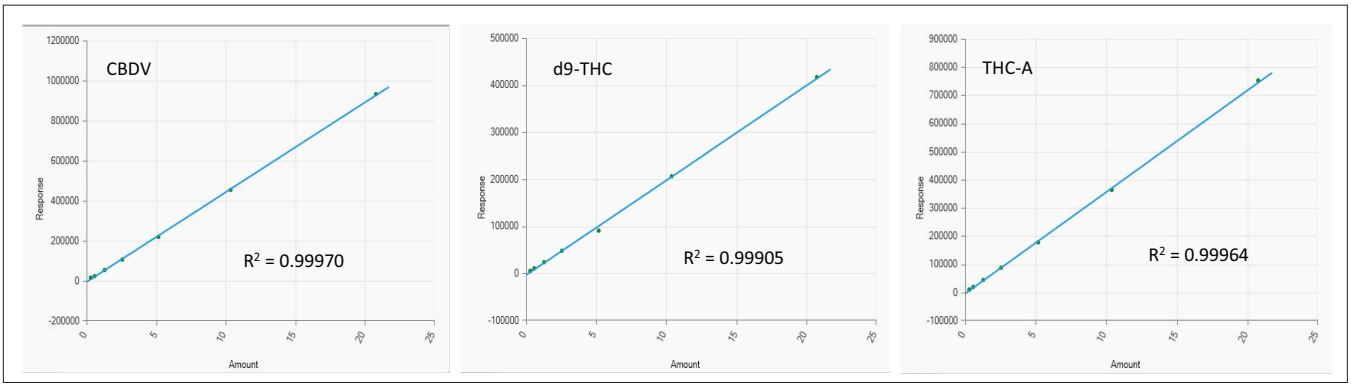


*Figure 4.* Linearity plots for CBDV, d9-THC and THC-A, concentration range: 0.33-20.8 µg/mL.

As listed in Table 3, LOQ (limit of quantitation) levels were established for each cannabinoid, based upon their averaged L1 calibration standard response (representative L1 chromatogram is shown in Figure 5). The calculated LOQs (≥10 S/N) were <0.04 µg/mL for all analyzed cannabinoids. Corrected for sample dilution, all LOQs were below 2.9 µg/mL. As cannabinoids in edibles are typically present in ≥ µg/mL (ppm) levels, these LOQs are well below the current concentrations of interest for the monitoring of cannabinoids in edible foods. It should be noted that, moving forward, if even lower LOQs are required, the Flexar PDA Plus's optional 50-mm flow cell would allow for this.

The overlaid chromatograms of the Chocolate Bar extract and the L4 standard are displayed in Figures 6, showing the close retention time match for the cannabinoids that were present. For clarity, only the more prominent analytes in the sample extract were labeled.

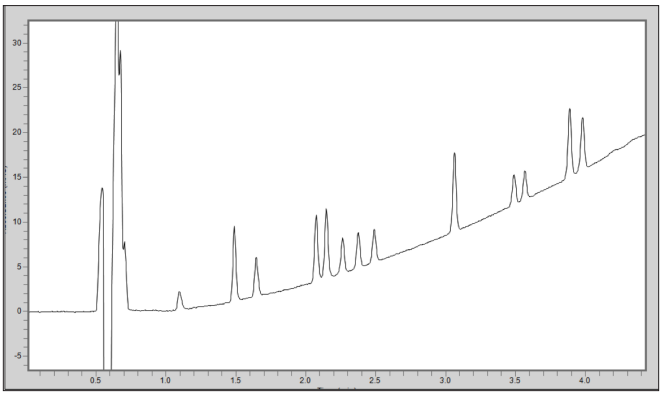

*Figure 5.* Chromatogram of the L1 standard (0.33 µg/mL).

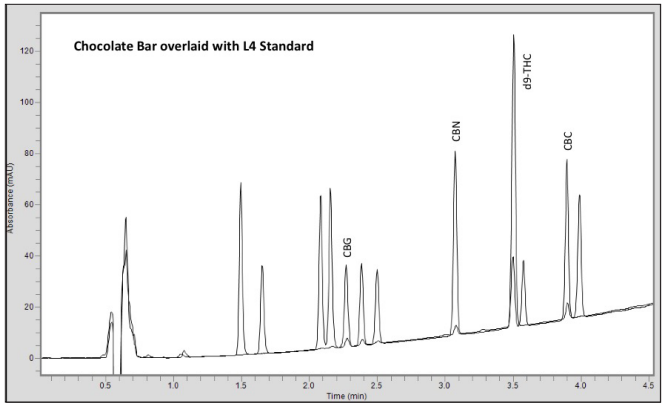


*Figure 6.* Overlaid chromatograms of the Chocolate Bar extract and the L4 standard.

*Table 3.* LOQs for the twelve cannabinoids.

| Cannabinoid | Calculated LOQ (µg/mL) | LOQ, corrected for dilution** ppm (Wgt/Wgt) | | | |
|---|---|---|---|---|---|
| | | A | B | C | D |
| Cannabidivarin (CBDV) | 0.013 | 0.83 | 1.04 | 2.08 | 16.64 |
| Cannabidivarinic Acid (CBDVA) | 0.026 | 1.66 | 2.08 | 4.16 | 33.28 |
| Cannabidiolic acid (CBDA) | 0.015 | 0.96 | 1.20 | 2.40 | 19.20 |
| Cannabigerol (CBG) | 0.028 | 1.79 | 2.24 | 4.48 | 35.84 |
| Cannabigerolic Acid (CBGA) | 0.032 | 2.04 | 2.56 | 5.12 | 40.96 |
| Tetrahydrocannabivarin (THCV) | 0.015 | 0.96 | 1.20 | 2.40 | 19.20 |
| Cannabidiol (CBD) | 0.029 | 1.86 | 2.32 | 4.64 | 37.12 |
| Cannabinol (CBN) | 0.013 | 0.83 | 1.04 | 2.08 | 16.64 |
| Δ9-Tetrahydrocannabinol (d9-THC) | 0.033 | 2.11 | 2.64 | 5.28 | 42.24 |
| Δ8-Tetrahydrocannabinol (d8-THC) | 0.036 | 2.30 | 2.88 | 5.76 | 46.08 |
| Cannabichromene (CBC) | 0.015 | 0.96 | 1.20 | 2.40 | 19.20 |
| Δ9-Tetrahydrocannabinolic acid (THC-A) | 0.019 | 1.22 | 1.52 | 3.04 | 24.32 |

**Corrected LOQs for: **A** = Cookie, Brownie, Rice Crispy Treat, Gummy1; **B** = Cherry Elixir, Sour Spray; **C** = Gummy2, Hard Candy, Lip Balm; **D** = Chocolate Bar

Figure 7 shows the chromatograms of the four more distinctive sample extracts that were analyzed. Most notable was the relatively high CBD content, as well as the significantly lower d9-THC content, in the Lip Balm. Characterization of the unknown peaks was not further pursued in this study.

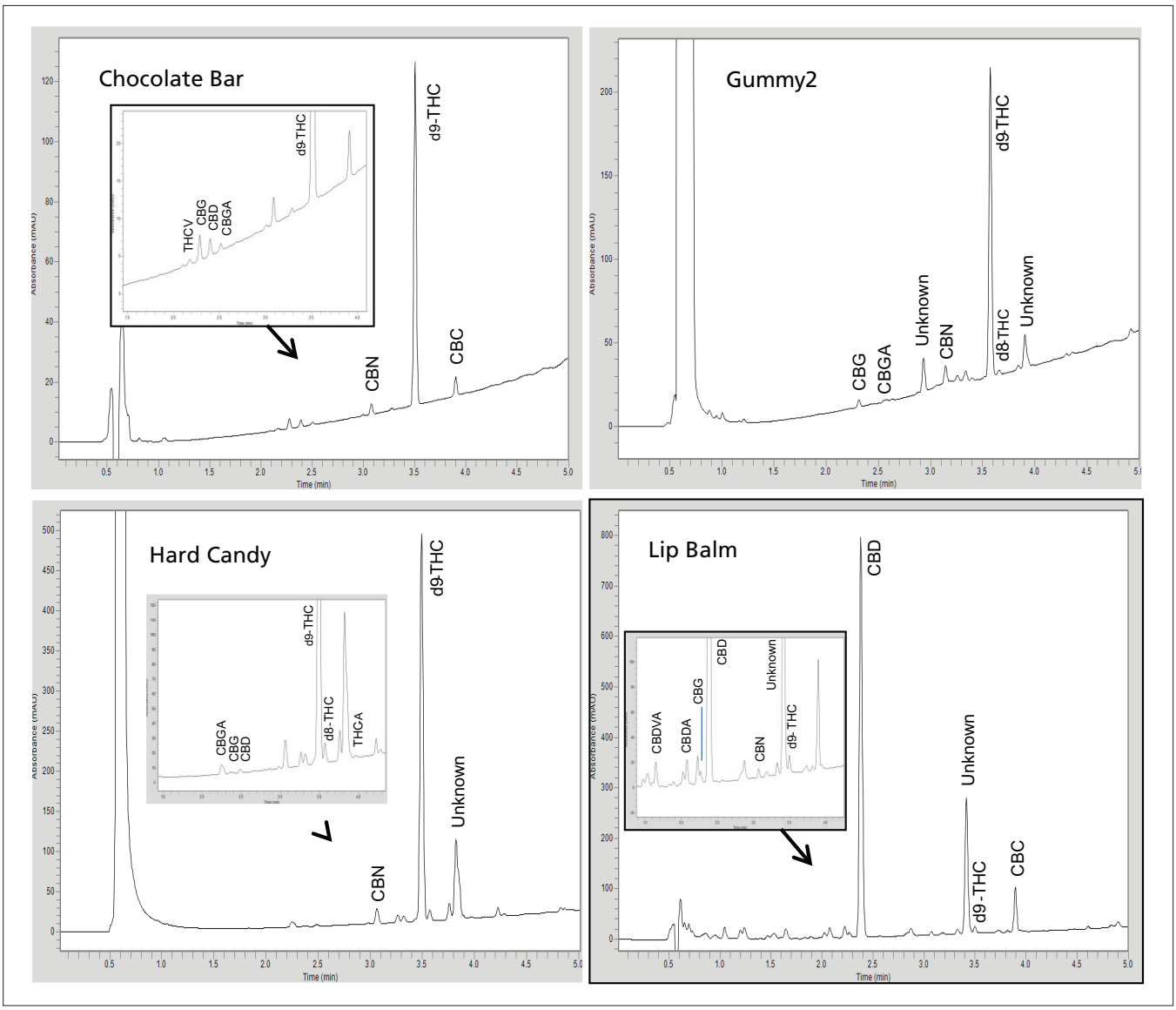


*Figure 7.* Chromatograms of the four more distinctive sample extracts.

To check for possible analyte carryover or background interference, a 80:20 methanol/water "blank" was run in triplicate, both after the calibration set and after the samples. In all cases, no carryover was observed for any of the analytes. A representative chromatogram of a blank injected after multiple sample injections is shown in Figure 8. No discernable peaks were found within the 1.5 to 4 minute region in which the cannabinoids eluted.

One interesting point of note, during the method development, it was found that a careful balance of the formic acid content between the water and acetonitrile mobile phases was able to reduce the baseline ramp. Specifically, after the 0.1 % formic was added to each mobile phase, adding another 200 uL of formic acid to the liter of acidified water significantly reduced the ramp, without any apparent adverse effect on peak resolution. As the resulting moderate ramp posed no issue and the

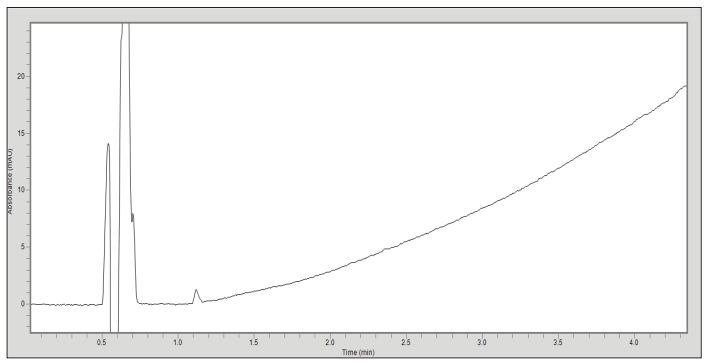


*Figure 8.* Chromatogram of the 80:20 methanol/water "blank" injection after sample injections.

chromatography was quite good at this point, further ramp improvement was not pursued during this study. However, if desired, it is quite likely that one could reduce the ramp even further, though one must be careful not to lose the chromatographic resolution of the cannabinoids.

Table 4 shows the calculated concentrations (µg/mL) for the twelve cannabinoids found in each of the ten sample extracts. It was noted that the Chocolate Bar had significantly higher d9-THC content and that the Lip Balm had significantly higher CBD content than all the other samples. It was also interesting that only the Gummy2 and the Hard Candy were found to contain quantitatable amounts of d8-THC, the analog of the more naturally prevalent d9-THC.

*Table 4.* Cannabinoid concentrations found in each of the ten edible food samples (average of three injections).

| Sample Extract | Analytes | Sample Concentration, Back-calculated for Extraction/Dilution (ug/g) | Analytes | Sample Concentration, Back-calculated for Extraction/Dilution (ug/g) |
|---|---|---|---|---|
| Cookie | CBDV | Trace | CBGA | 13.1 |
| | CBDVA | ND | CBN | 14.3 |
| | CBDA | ND | d9-THC | 965.5 |
| | THCV | ND | d8-THC | ND |
| | CBG | 32.2 | CBC | 22.9 |
| | CBD | Trace | THC-A | Trace |
| Chocolate Bar | CBDV | ND | CBGA | 229.4 |
| | CBDVA | ND | CBN | 254.1 |
| | CBDA | Trace | d9-THC | 12913.7 |
| | THCV | 149.0 | d8-THC | ND |
| | CBG | 438.2 | CBC | 431.3 |
| | CBD | 339.7 | THC-A | Trace |
| Brownie | CBDV | Trace | CBGA | 10.8 |
| | CBDVA | ND | CBN | 20.3 |
| | CBDA | ND | d9-THC | 864.7 |
| | THCV | ND | d8-THC | ND |
| | CBG | 25,5 | CBC | 18.7 |
| | CBD | Trace | THC-A | ND |
| Rice Crispy Treat | CBDV | 12.7 | CBGA | 19.1 |
| | CBDVA | Trace | CBN | 26.7 |
| | CBDA | ND | d9-THC | 1853.5 |
| | THCV | 14.3 | d8-THC | Trace |
| | CBG | 37.3 | CBC | 38.1 |
| | CBD | Trace | THC-A | 13.6 |
| Gummy1 | CBDV | Trace | CBGA | 13.3 |
| | CBDVA | ND | CBN | 14.7 |
| | CBDA | ND | d9-THC | 1037.3 |
| | THCV | 11.6 | d8-THC | ND |
| | CBG | 23.3 | CBC | 20.8 |
| | CBD | Trace | THC-A | ND |
| Gummy2 | CBDV | 18.3 | CBGA | 44.8 |
| | CBDVA | ND | CBN | 71.5 |
| | CBDA | ND | d9-THC | 2703.8 |
| | THCV | Trace | d8-THC | 50.4 |
| | CBG | 70.1 | CBC | ND |
| | CBD | 20.1 | THC-A | ND |
| Hard Candy | CBDV | ND | CBGA | 57.6 |
| | CBDVA | ND | CBN | 142.7 |
| | CBDA | ND | d9-THC | 8069.8 |
| | THCV | ND | d8-THC | 239.0 |
| | CBG | 152.5 | CBC | ND |
| | CBD | 44.8 | THC-A | 38.0 |
| Lip Balm | CBDV | 34.6 | CBGA | 16.9 |
| | CBDVA | 162.0 | CBN | 27.1 |
| | CBDA | 83.0 | d9-THC | 114.7 |
| | THCV | 87.7 | d8-THC | ND |
| | CBG | 80.6 | CBC | 299.8 |
| | CBD | 5583.5 | THC-A | 14.8 |
| Cherry Elixir | CBDV | ND | CBGA | 12.0 |
| | CBDVA | ND | CBN | 16.3 |
| | CBDA | ND | d9-THC | 805.4 |
| | THCV | ND | d8-THC | ND |
| | CBG | 24,2 | CBC | 19.7 |
| | CBD | ND | THC-A | Trace |
| Sour Spray | CBDV | ND | CBGA | 12.2 |
| | CBDVA | ND | CBN | 15.3 |
| | CBDA | ND | d9-THC | 782.5 |
| | THCV | ND | d8-THC | ND |
| | CBG | 23.8 | CBC | 19.1 |
| | CBD | Trace | THC-A | 11.8 |

ND = Not detected

## Conclusion

- This work has demonstrated the effective chromatographic separation and quantitation of twelve cannabinoids, including THC and THC-A, in edible food extracts using the PerkinElmer Flexar HPLC system with a photodiode array detector.
- The method provides exceptional chromatographic repeatability and affords LOQs well below the current concentration levels of interest for cannabinoids in edibles.
- Thereupon, the method/procedure defined herein can be expected to fulfill the essential task of ensuring product uniformity and cannabinoid screening in edible foods.

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