

The Potency Determination of 15 Cannabinoids using the Cannabis Analyzer for Potency™

■ Introduction

Revenue for cannabis testing is set to rise to roughly 2 billion USD by 2025. According to a 2019 Global Market Insights, Inc¹, the chromatography technology segment will account for 1.5 billion USD of this revenue. The potential for market growth is attributed to the ongoing development of chromatography techniques in potency testing of cannabis¹, supporting manufacturing operations, and the associated clinical science. Cannabis is available in numerous forms, from dry flower to concentrated oils, and contains over one hundred cannabinoids, making the development of rugged, quantitatively accurate methods a challenge. This study optimizes a quantitative chromatographic determination of 15 cannabinoids using the Shimadzu Cannabis Analyzer for Potency™.

■ Equipment and Method

For this study a Shimadzu Cannabis Analyzer for Potency™ – an integrated HPLC system with built-in UV detector – was used. Table 1 shows the instrument and method parameters summary. To create a 100.0 µg/mL mixture consisting of 15 components mixture. A mixture of 11 cannabinoids (CRM; PN: 220-91239-21) was supplemented with four additional cannabinoid standards (Cerilliant).

Table 2 shows a list of initial concentrations for each standard. Quality Control (QC) standards were prepared using the same method as the calibration standards.

Table 2: Initial concentrations for the 15 cannabinoids prior to mixture preparation

| No. | Standard | Compounds | Stock Conc. (mg/L) |
|-----|------------|-----------|--------------------|
| 1 | Shimadzu | CBDV | 250 |
| 2 | Shimadzu | CBDA | 250 |
| 3 | Shimadzu | CBGA | 250 |
| 4 | Shimadzu | CBG | 250 |
| 5 | Shimadzu | CBD | 250 |
| 6 | Shimadzu | THCV | 250 |
| 7 | Shimadzu | CBN | 250 |
| 8 | Shimadzu | d9-THC | 250 |
| 9 | Shimadzu | d8-THC | 250 |
| 10 | Shimadzu | CBC | 250 |
| 11 | Shimadzu | THCA | 250 |
| 12 | Cerilliant | CBDVA | 1000 |
| 13 | Cerilliant | THCVA | 1000 |
| 14 | Cerilliant | CBL | 1000 |
| 15 | Cerilliant | CBCA | 1000 |

Table 1: Summary of method and instrument parameters

| Item | Description |
|---------------------------|---|
| HPLC System | Cannabis Analyzer for Potency™, 220-94420-00 |
| Detector | UV-Vis |
| Wavelength Monitored (nm) | 220 |
| Mobile Phase A | 0.085% Phosphoric Acid in Water |
| Mobile Phase B | 0.085% Phosphoric Acid in Acetonitrile |
| Gradient Program | 70% B for 3 min; 70%-85% B over 6 min; 85%-95% B over 0.01 min; 95% B for 0.99 min; 95%-70% B over 0.01 min; 70% B for 4.99 min |
| Column | NexLeaf CBX for Potency, 2.7 µm, 4.6 x 150 mm column, 220-91525-70 |
| Guard column | NexLeaf CBXGuard Column Cartridge, 220-91525-72 |
| Flowrate (mL/min) | 1.6 |
| Oven Temperature (°C) | 35 |
| Injection Volume (µL) | 5 |

■ Results and Discussion

A six-point calibration curve ranging from 0.5 to 100 µg/mL and three Quality Control (QC) standards, 2.5 µg/mL, 25 µg/mL and 75 µg/mL, were prepared. Calibration curves and QC standards were evaluated using seven replicate injections and evaluating the correlation coefficient (R^2) of the linear regression. All calibration curves passed the high-sensitivity method criteria ($R^2 \geq 0.999$).

Figure 1 shows the calibration curves for the 15 target cannabinoids. A best-fit weighting method (1/C) was selected for the linear regression for calibration curve quantitation. The statistical results were processed via Browser in LabSolutions Database, version 6.83; results are shown in table 3.

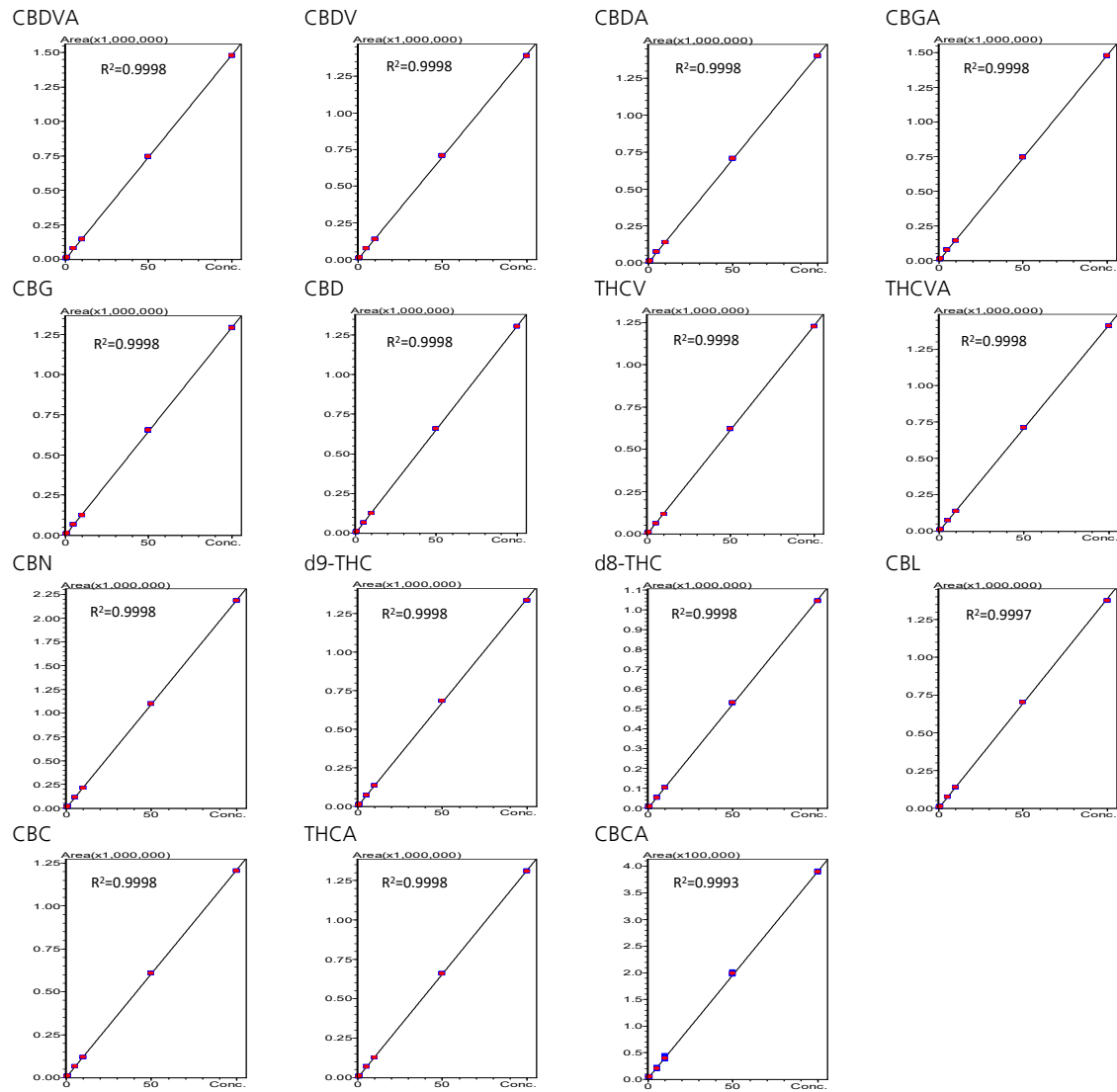


Figure 1: Standard curves for 15 cannabinoids

Table 3: Statistical analysis of 6-point calibration curve with seven replicates for calibration standards and quality control (QC) standards for the 15-cannabinoid mixture

| No. | Compound | Calibration Results | | 2.5 ppm (QC Low) | | | 25.0 ppm (QC Medium) | | | 75.0 ppm (QC High) | | |
|-----|----------|---------------------|----------------|------------------|---------|--------------|----------------------|---------|--------------|--------------------|---------|--------------|
| | | RF RSD (%) | R ² | Mean Conc. | RSD (%) | Accuracy (%) | Mean Conc. | RSD (%) | Accuracy (%) | Mean Conc. | RSD (%) | Accuracy (%) |
| 1 | CBDVA | 3.950 | 0.9998 | 2.62 | 1.265 | 104.6 | 25.38 | 0.206 | 101.5 | 77.18 | 0.665 | 102.9 |
| 2 | CBDV | 5.002 | 0.9998 | 2.63 | 1.031 | 105.4 | 25.74 | 0.235 | 102.9 | 77.83 | 0.577 | 103.8 |
| 3 | CBDA | 4.320 | 0.9998 | 2.62 | 1.150 | 103.6 | 25.45 | 0.233 | 101.8 | 77.17 | 0.699 | 102.9 |
| 4 | CBGA | 4.372 | 0.9998 | 2.59 | 0.904 | 101.7 | 25.44 | 0.137 | 101.8 | 76.81 | 0.668 | 102.4 |
| 5 | CBG | 6.721 | 0.9998 | 2.54 | 0.708 | 98.4 | 25.49 | 0.201 | 102.0 | 78.06 | 0.616 | 104.1 |
| 6 | CBD | 4.637 | 0.9998 | 2.46 | 0.689 | 100.4 | 25.45 | 0.250 | 101.8 | 78.20 | 0.742 | 104.3 |
| 7 | THCV | 4.836 | 0.9998 | 2.51 | 0.766 | 105.1 | 25.50 | 0.198 | 102.0 | 77.82 | 0.512 | 103.8 |
| 8 | THCVA | 3.557 | 0.9998 | 2.63 | 0.772 | 105.1 | 25.50 | 0.287 | 102.0 | 76.38 | 0.783 | 101.8 |
| 9 | CBN | 3.587 | 0.9998 | 2.63 | 0.603 | 98.5 | 25.41 | 0.226 | 101.6 | 77.83 | 0.659 | 103.8 |
| 10 | d9-THC | 9.869 | 0.9998 | 2.46 | 5.300 | 100.0 | 25.82 | 0.383 | 103.3 | 77.96 | 0.531 | 103.9 |
| 11 | d8-THC | 6.941 | 0.9998 | 2.50 | 5.455 | 108.3 | 25.85 | 0.493 | 103.4 | 77.88 | 0.741 | 103.8 |
| 12 | CBL | 6.867 | 0.9997 | 2.71 | 2.585 | 108.3 | 25.85 | 0.532 | 103.4 | 77.68 | 0.631 | 103.6 |
| 13 | CBC | 9.092 | 0.9998 | 2.60 | 2.838 | 104.0 | 25.48 | 0.370 | 101.9 | 77.97 | 0.629 | 104.0 |
| 14 | THCA | 9.222 | 0.9998 | 2.70 | 3.609 | 107.9 | 25.55 | 0.359 | 102.2 | 76.57 | 0.699 | 102.1 |
| 15 | BCA | 38.577 | 0.9993 | 2.28 | 13.645 | 91.0 | 25.78 | 3.193 | 103.1 | 75.69 | 2.017 | 100.9 |

For the noise/drift calculations as well as detection limit and quantitation limit (Table 4), we selected a specified range from 1.20 min to 2.20 min using the ASTM calculation method. Limits of Detection (LOD) and Quantitation (LOQ) of 3.3 and 10.0 were selected, respectively. LOD and LOQ are terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure.

By using the signal-to-noise method, the peak-to-peak noise around the analyte retention time was measured. A signal-to-noise ratio (S/N) of three is generally accepted for estimating LOD and signal-to-noise ratio of ten is used for estimating LOQ. This method is commonly applied to analytical chromatographic methods.²⁸³

Table 4: Detection limit and quantitative limit for 15 components at 0.5 µg/mL

| ID# | Name | S/N | Detection Limit (LOD) | Quantitative Limit (LOQ) |
|-----|--------|-------|-----------------------|--------------------------|
| 1 | CBDVA | 13.68 | 0.12 | 0.37 |
| 2 | CBDV | 12.60 | 0.13 | 0.39 |
| 3 | CBDA | 10.95 | 0.15 | 0.45 |
| 4 | CBGA | 11.09 | 0.15 | 0.46 |
| 5 | CBG | 9.83 | 0.18 | 0.54 |
| 6 | CBD | 8.70 | 0.20 | 0.60 |
| 7 | THCV | 8.22 | 0.20 | 0.62 |
| 8 | THCVA | 9.85 | 0.17 | 0.51 |
| 9 | CBN | 16.13 | 0.10 | 0.31 |
| 10 | d9-THC | 10.58 | 0.15 | 0.47 |
| 11 | d8-THC | 7.56 | 0.23 | 0.70 |
| 12 | CBL | 10.23 | 0.16 | 0.49 |
| 13 | CBC | 9.73 | 0.18 | 0.53 |
| 14 | THCA | 8.34 | 0.18 | 0.56 |
| 15 | BCA | 3.39 | 0.72 | 2.18 |

Figure 2 shows a representative chromatogram for three QC standards. Figure 3 illustrates an overlaid chromatogram of seven injections at 100 ppm for the 15-cannabinoid mixture.

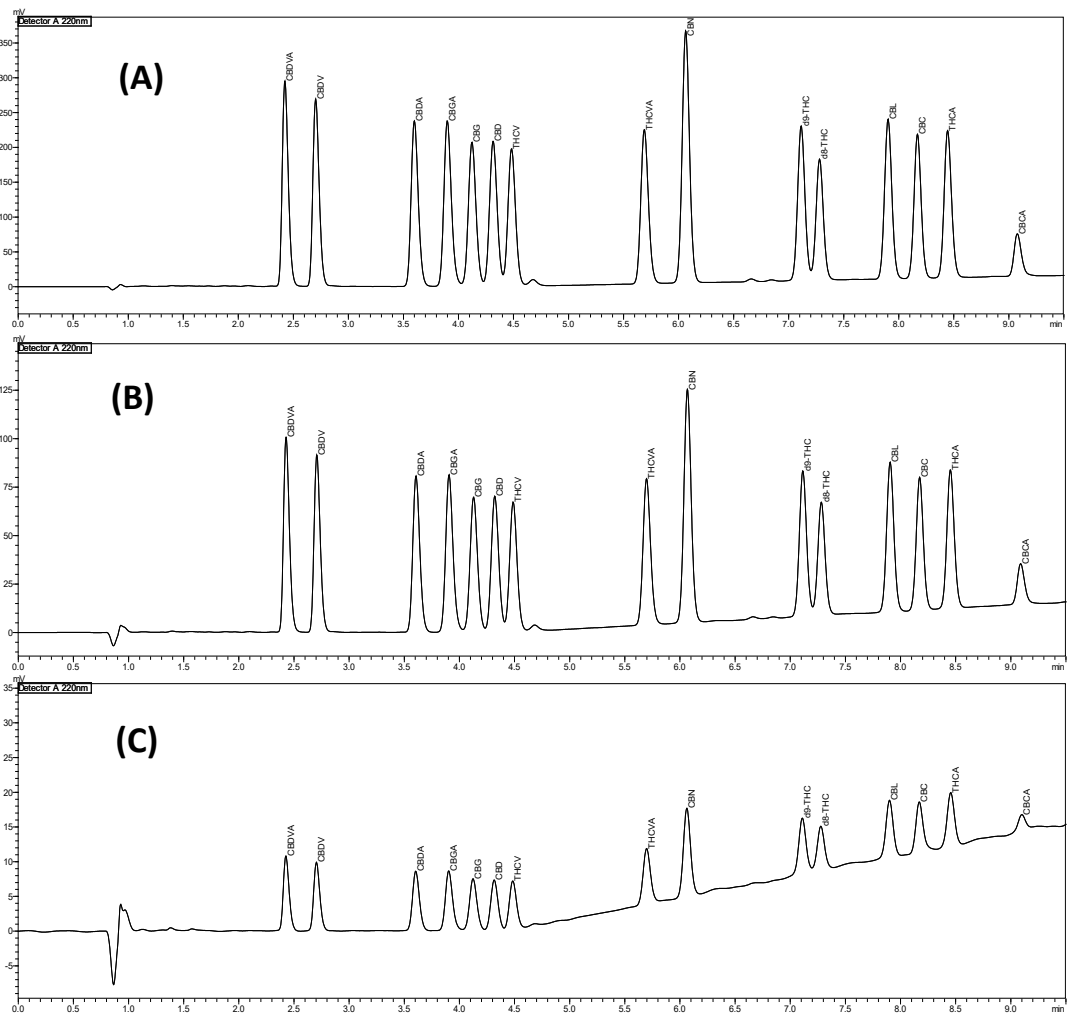


Figure 2: A representative chromatogram showing quality control standards. A 5 μ L of (A) QC high, (B) QC medium and (C) QC low

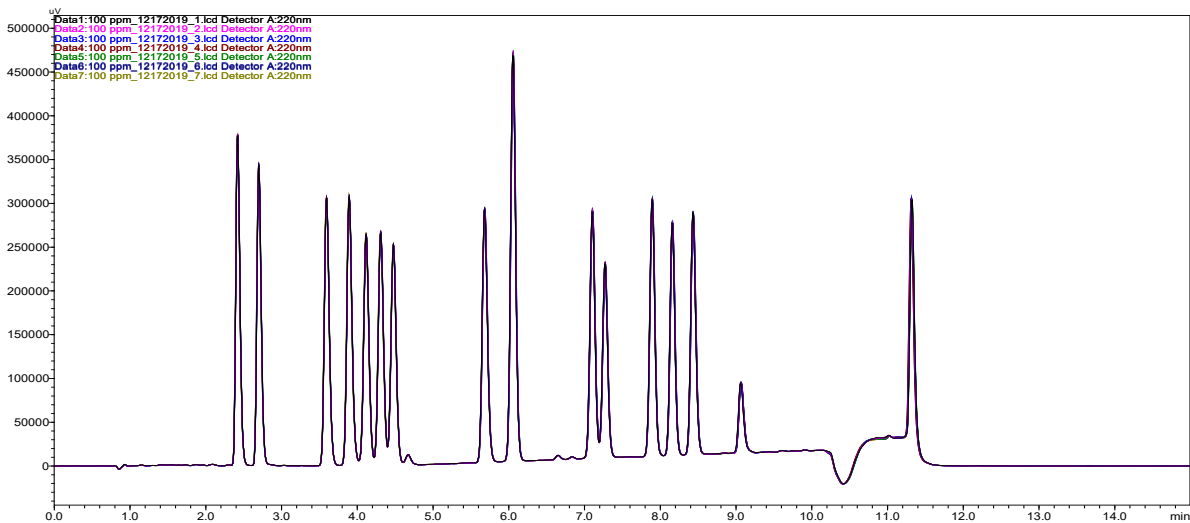


Figure 3: The 15-cannabinoid mixture - Overlay of seven injections (5 μ L injection at 100 ppm)

■ Conclusion

In response to the increasing demand for development of chromatography techniques in potency testing of cannabis and hemp, we developed a method that builds on the existing High Sensitivity Method using the Shimadzu Cannabis Analyzer for Potency™, optimized for the quantitative determination of 15 major cannabinoids. The statistical results document rigorous testing for retention time and peak area repeatability, quantitative accuracy and sensitivity.

■ References

1. <https://www.globenewswire.com/news-release/2019/07/16/1883110/0/en/Cannabis-Testing-Market-to-surpass-USD-2-Billion-by-2025-Global-Market-Insights-Inc.html>.
 2. D.A. Armbruster, M.D. Tillman and L.M. Hubbs. Limit of detection (LOD)/limit of quantitation (LOQ): comparison of the empirical and the statistical methods exemplified with GC-MS assays of abused drugs. *Clinical Chemistry*. 1994; 40(1): 1233-8.
 3. Shrivastava and V.B. Gupta. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Review Article*. 2011; 2(1): 21-5.
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