# Analytical Data Confidence is Highest with Commercially Prepared CRM Mixtures

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High-quality reference materials with established metrological traceability are an essential element for measurement accuracy during routine analytical checks as well as for cannabis product quality testing and profiling. We compare two different methods using certified reference materials (CRMs) to generate a standard for the quantitation of 10 prevalent phytocannabinoids against a calibration curve to show that premade multicomponent CRM mixtures improve quantitation accuracy.

With the increased legalization of cannabis products, various stakeholders are moving toward standardizing this growing industry as a way to protect consumers. As part of this standardization, cannabis testing laboratories are strongly urged, if not required, to become ISO/IEC 17025 accredited. A key component for testing under an ISO/IEC 17025 quality system is the use of a certified reference material (CRM) to maintain metrological traceability in its measurements (1). ISO 17034 commercially produced CRMs are ideal for quantitative analysis in the cannabis testing field. A CRM's certificate of analysis will include a statement of metrological traceability as well as the certified concentration with an associated measurement uncertainty of the material.

Historically, single analyte CRMs have been the default option for quality control testing, mainly due to the lack of availability of multicomponent CRMs. Now that multicomponent CRMs are available, they provide an advantage in many applications. For instance, when evaluating several analytes at once, a commercially formulated multicomponent CRM bypasses the additional steps an analyst must take to create a stock solution from single CRMs. The data provided in this application note compares a typical method for preparing stock solutions from several single-component CRMs (Method A) against the use of a premade multicomponent CRM (Method B).

## Methods

Mixtures created from each of these methods were injected on the Cannabis Analyzer for Potency™ HPLC model LC-2030C Plus from Shimadzu Scientific Instruments. Concentrations of 1 µg/mL and 100 µg/mL were analyzed against the weighted calibration curve to evaluate differences between the two methods. For brevity, the 100 µg/mL results are discussed here; the full application note may be found at www.caymanchem.com/CRMmixtures. Accuracy of the mixtures was determined by comparing the experimental concentrations to the theoretical concentrations of each component.

The calibration curve was developed using Cayman's Phytocannabinoid Mixture 10 (CRM) (Cayman Item No. 21305) in accordance with the "Cannabis Analyzer for Potency™ Quick Guide" provided by Shimadzu (2). This mixture, in acetonitrile, contains 250 µg/mL of each of the following: cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabidiol (CBD), cannabinol (CBN), tetrahydrocannabinolic acid A (THCA-A), $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), $\Delta^8$-tetrahydrocannabinol ($\Delta^8$-THC), and (±)-cannabichromene (CBC). The high-resolution method from Shimadzu was used in the analysis of this calibration curve (3). A linear dynamic range of 0.5 µg/mL to 250 µg/mL was established for each of the 10 analytes in the Phytocannabinoid Mixture 10 (CRM). A weighted regression curve of $1/[X]^2$ was generated for each compound to provide a relative representation of concentration at both the low and high ends of the curve.

For Method A, a stock solution containing 10 single component CRMs (each at 1 mg/mL in 1 mL of either acetonitrile or methanol) was prepared by pouring each of the single CRMs into separate HPLC vials. Then, 1 mL of each individual CRM compound was pipetted into a scintillation vial to

**Table I:** Accuracy, relative error, and precision ($n$ = 8)

| 100 µg/mL | Accuracy (%) | | Relative Error (%) | | Precision (CV, %) | |
|---|---|---|---|---|---|---|
| Compound | Method A | Method B | Method A | Method B | Method A | Method B |
| CBDV | 102.77 | 97.14 | 2.77 | -2.86 | 3.72 | 0.60 |
| CBDA | 98.71 | 99.10 | -1.29 | -0.90 | 7.14 | 0.63 |
| CBGA | 104.94 | 98.24 | 4.94 | -1.76 | 3.58 | 0.66 |
| CBG | 101.42 | 96.55 | 1.42 | -3.45 | 5.44 | 0.64 |
| CBD | 97.72 | 95.32 | -2.28 | -4.68 | 1.49 | 0.61 |
| CBN | 105.31 | 101.43 | 5.31 | 1.43 | 2.99 | 0.62 |
| THCA-A | 103.45 | 99.21 | 3.45 | -0.79 | 2.53 | 0.64 |
| $\Delta^9$-THC | 98.24 | 98.40 | -1.76 | -1.60 | 4.80 | 0.61 |
| $\Delta^8$-THC | 103.40 | 100.46 | 3.40 | 0.46 | 2.85 | 0.58 |
| CBC | 100.52 | 100.02 | 0.52 | 0.02 | 2.64 | 0.64 |

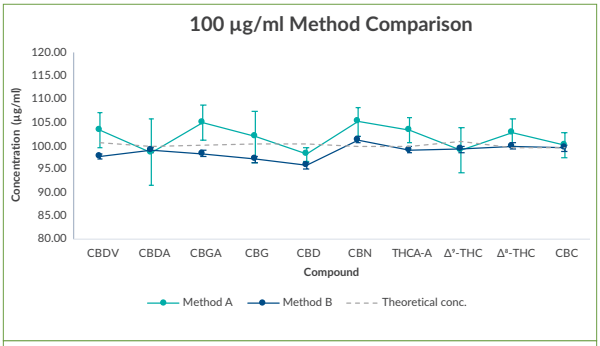


**Figure 1:** Experimental concentration at 100 µg/mL of Method A and Method B solutions compared to respective theoretical concentrations.

create a mixture at a concentration of 100 µg/mL. For Method B, an independent batch of Cayman's premade Phytocannabinoid Mixture 10 (CRM) served as the commercially formulated multicomponent CRM. This was poured into a separate HPLC vial, then a 400 µL aliquot was transferred to a 1 mL volumetric flask and brought to volume with methanol to create the 100 µg/mL solution. On two separate days, eight solutions were prepared using each method by two separate analysts.

## Results and Discussion

For both methods, a single injection of each solution ($n$ = 8) was analyzed, generating a total of 16 injections. Two samples, one each from Method A and Method B, were analyzed on a separate day than the other 14 samples. The experimental concentration for each component was derived from the calibration curve. These concentrations were averaged across all injections for each component, plotted with their respective standard deviations, and compared to the theoretical concentrations (Figure 1). The theoretical concentrations were calculated based on the certified concentrations from the certificates of analysis.

The accuracy, relative error, and precision results were tabulated for each compound (Table I). The accuracy and relative error of the experimental concentrations for each of the sample components were calculated against the respective theoretical concentrations. Greater deviation of the concentrations was observed with the Method A solution than with the Method B solution. Some variations in both Method A and B may be attributed to the loss of solution during the transfer of the CRM to the larger HPLC vial, altering the concentration. A relative error of ±10% of the verified concentration is an acceptable criterion in analytical testing (4). Using this value, the calculated relative error for both Method A and Method B meet this criterion. We defined precision as the coefficient of variation in percent form. As shown in Table I, the precision observed by Method B is better across all components compared to Method A.

## Conclusions

There are multiple approaches to create working solutions of a phytocannabinoid mixture. Complications can arise when preparing a stock standard using single-component CRMs. All CRM materials used in this experiment were produced under an ISO 17034 quality system and, therefore, had metrologically traceable concentrations before the ampules were opened. The variability observed in the experimental concentrations of the Method A solution was likely related to the preparation of the stock solution. The multiple pipetting steps required to prepare the mixture may have affected the actual concentration of the stock solution. Furthermore, each preparation event, such as pipetting, adds uncertainty to the reported value and may also compromise the metrological traceability of the concentration when each component is removed from its original container and added to the mixture.

While the approach utilized in Method A is a seemingly practical method seen in industry standards, inconsistencies in reported concentrations were noted across multiple injections in our experiment. The commercially prepared CRM mixture was shown to improve precision and accuracy of the analysis. When used with proper methodology, ISO 17034-produced multicomponent CRM mixtures are designed to give the user confidence in their analytical data. They also offer a simpler approach, saving time, effort, and cost.

## References

1) International Standard ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories (2017).

2) Shimadzu, "Cannabis analyzer for potency quick guide" (2018).

3) Shimadzu, "Potency testing in cannabis extracts using a high-resolution method with the cannabis analyzer for potency" (2017).

4) B. DeSilva, W. Smith, R. Weiner, et al. *Pharm. Res.* **20**(11), 1885–1900 (2003).