Application Note

Comparison of CRM Concentrations Among Four Different Reference Material Producers

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Key Features

- ISO-certified CRMs from multiple manufacturers are employed by analytical facilities to identify *Cannabis* components and report potency values.
- Reference material consistency across vendors is needed to ensure accuracy of reporting.
- We compare the reported concentration of 11 individual cannabinoid CRMs from four different reference material producers.
- No significant variability in CRM concentrations exists among products produced under the ISO 17034 standard, reaffirming the competence of manufacturers

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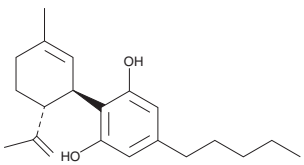
Introduction

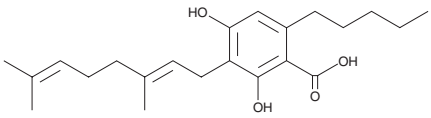
One of the hurdles facing the *Cannabis* industry is standardization of analytical methods to ensure accurate identification of plant content and consistent potency value reporting. As testing, calibration, and sampling facilities rely on commercially manufactured analytical standards as part of their quality control measures, the quality of these reference materials will undoubtedly influence data reporting. Rigorous quality systems accredited under ISO/IEC 17025 establish this assurance by using properly calibrated equipment and, when available, certified reference materials (CRMs) that are produced by reference material producers accredited to ISO 17034—the international standard governing general requirements for the competence of reference material producers.

CRMs are available as single components or as pre-made mixtures of components, which are useful when examining multiple elements in a single sample. Although a pre-made CRM mixture lends to efficiencies in sample preparation, analytical laboratories may also use single CRMs to formulate customized mixtures. Both approaches will maintain ISO 17034 standards for metrological traceability as long as proper dilution techniques are observed.[1,2] Once primary data is obtained, analytical facilities may require a secondary source of reference materials for data confirmation. Based on the accrediting body's regulation, this secondary source can either be a different batch number from the same vendor or a batch obtained from a second vendor (if an alternate vendor is available). After a laboratory's analytical method is validated, CRMs are routinely used to ensure ongoing analytical accuracy. There will be batch-to-batch variability between CRMs from the same supplier or between suppliers, but due to the rigor of ISO 17034 standards, all ampules should be within a tolerable deviation of the target concentration so these standards should be inherently interchangeable.

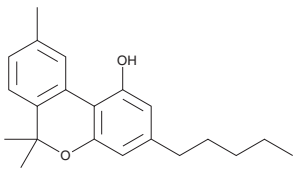
We compared the reported concentration of 11 individual cannabinoid CRMs from four different vendors to a pre-made phytocannabinoid CRM mixture to identify if significant variability exists among reference material producers. The phytocannabinoids selected were based on the most commonly tested phytocannabinoid components in *Cannabis* samples: cannabidiol (CBD), cannabigerolic acid (CBGA), cannabinol (CBN), cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabigerol (CBG), tetrahydrocannabinolic acid A (THCA-A), Δ^8-tetrahydrocannabinol (Δ^8-THC), tetrahydrocannabivarin (THCV), Δ^9-tetrahydrocannabinol (Δ^9-THC), and (±)-cannabichromene (CBC). Structures are noted below. This work was done using individual CRMs from each supplier to replicate the approach that is routinely undertaken in analytical testing laboratories.



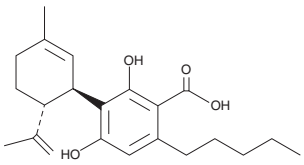
CBD



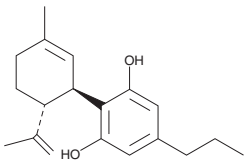
CBGA



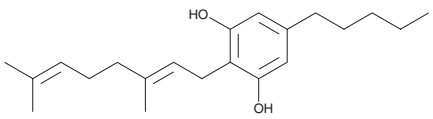
CBN



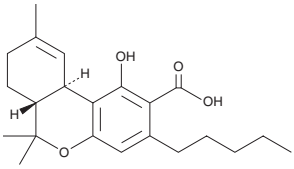
CBDA



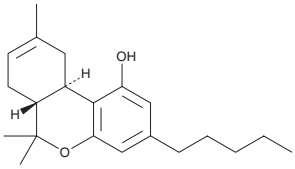
CBDV



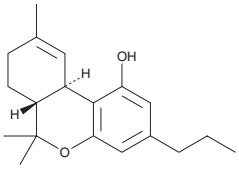
CBG



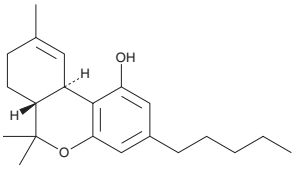
THCA-A



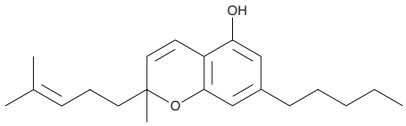
Δ^8-THC



THCV



Δ^9-THC



CBC

Methods

Single CRMs for CBD, CBGA, CBN, CBDA, CBDV, CGB, THCA-A, Δ^8-THC, THCV, Δ^9-THC, and CBC were obtained from four different reference material producers and blinded as vendors A-D. These selected CRMs were used to replicate the routine testing that is performed in laboratories after a method is validated. All materials were stored at the temperatures recommended in each individual certificate of analysis (CofA). Prior to sample preparation, ampules were set on the benchtop to attain room temperature. Each ampule was shaken thoroughly before use. A 0.25 mg/ml dilution of each of the 11 components was created by pipetting 25 µl of the 1.0 mg/ml sample into a separate HPLC vial fitted with a low volume insert. Then, 75 µl of acetonitrile was added to the low volume insert. The solution was mixed by pipetting the liquid up and down several times. The vial was capped and gently vortexed to ensure homogeneity.

Samples were placed in an Agilent Technologies 1100 Series HPLC for testing. All samples were made from freshly opened ampules and were prepared the same day as testing. A freshly opened ampule of a pre-made 11-component CRM mixture of phytocannabinoids commercially available from Shimadzu Scientific Instruments (Shimadzu Part Number: 220-91239-21; manufactured by Cayman Chemical) was used for each sequence. The sequence alternated the samples from all reference material producers with the 11-component mixture spaced in between at standard intervals so that a bias (drift) in the measurement could be separated from a trend in the sample. For each single component, an example of the analytical sequence order of replicates occurred as follows: vendor A (CBD), vendor B (CBD), vendor C (CBD), vendor D (CBD), 11-mix. The integration parameters were maintained for each analyte across all vendors. All samples were tested in triplicate. We defined a relative error of ±10% of the verified concentration as acceptable criterion in analytical testing.[3] This ±10% criterion was used to determine acceptable results for the purposes of this publication with the caveat that acceptance criteria may be defined differently elsewhere.

Results

The calculated concentration of each vendor CRM ($C_{calc\ vendor}$) was determined by comparing the area counts of the vendor material to the pre-made 11-component CRM mixture external standard. The concentration difference (C_{diff}) of the calculated concentration from the reported CofA concentration (C_{CofA}) was calculated for each component (C_{diff} = ($C_{calc\ vendor}$ /C_{CofA})*100%). The area counts for the individual component CRM concentrations from four different vendors all fell within ±10% of the pre-made 11-component mixture (**Figure 1** and **Table 1**). Variations observed are likely related to preparation events involving solution transfer, as pipetting can add uncertainty to a reported value. Nonetheless, these differences still fell within acceptable tolerances.

Reported Concentration Comparison

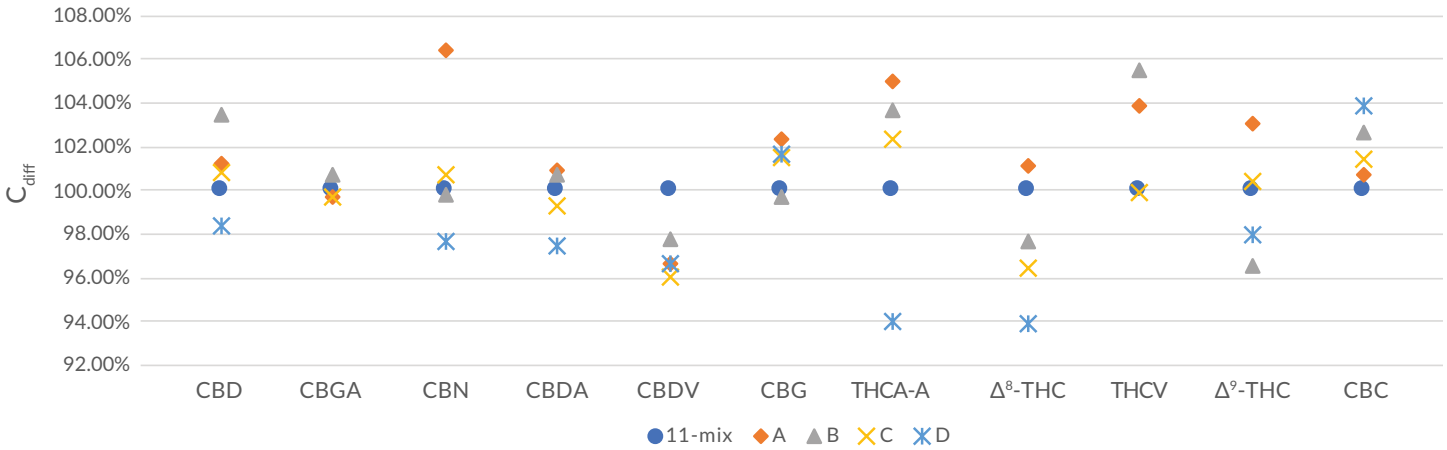


Figure 1. Calculated concentration difference (C_{diff}) of an 11-component CRM mixture (11-mix) compared to single CRM standards from vendors A-D.

A secondary confirmation was performed by calculating the concentration against the 6-point calibration curve created from the area counts of the standards. For this measurement, the area counts of the sample were entered into the equation for the line from the calibration curve, and the concentration was calculated. The calculated concentration was compared against the reported concentration on the sample's CofA. Single-component CRM concentrations also matched within ±10% of the concentration reported on the corresponding CofAs (data not shown).

Table 1. Calculated concentration difference (C_{diff}) of single CRM samples from vendors A-D versus an 11-component CRM mixture. NA = not available.

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
CBD	Concentration (mg/ml)				
	0.2504	0.2500	0.2500	0.2504	0.2510
	C_{diff} vs. 11-mix				
		101.3%	103.5%	100.9%	98.4%

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
THCA-A	Concentration (mg/ml)				
	0.2487	0.2500	0.2500	0.2506	0.2499
	C_{diff} vs. 11-mix				
		105.1%	103.7%	102.4%	94.0%

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
CBGA	Concentration (mg/ml)				
	0.2508	0.2500	0.2500	0.2500	NA
	C_{diff} vs. 11-mix				
		99.7%	100.7%	99.7%	NA

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
Δ^8-THC	Concentration (mg/ml)				
	0.2538	0.2500	0.2500	0.2510	0.2478
	C_{diff} vs. 11-mix				
		101.2%	97.7%	96.5%	94.0%

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
CBN	Concentration (mg/ml)				
	0.2541	0.2500	0.2500	0.2506	0.2490
	C_{diff} vs. 11-mix				
		106.4%	99.9%	100.7%	97.7%

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
THCV	Concentration (mg/ml)				
	0.2388	0.2500	0.2500	0.2500	NA
	C_{diff} vs. 11-mix				
		103.9%	105.6%	100.0%	NA

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
CBDA	Concentration (mg/ml)				
	0.2468	0.2500	0.2500	0.2505	0.2503
	C_{diff} vs. 11-mix				
		100.9%	100.8%	99.3%	97.5%

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
Δ^9-THC	Concentration (mg/ml)				
	0.2549	0.2500	0.2503	0.2500	0.2503
	C_{diff} vs. 11-mix				
		103.1%	96.6%	100.4%	98.1%

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
CBDV	Concentration (mg/ml)				
	0.2552	0.2500	0.2500	0.2508	0.2485
	C_{diff} vs. 11-mix				
		96.7%	97.8%	96.1%	96.7%

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
CBC	Concentration (mg/ml)				
	0.2487	0.2500	0.2500	0.2503	0.2525
	C_{diff} vs. 11-mix				
		100.8%	102.7%	101.5%	103.9%

	11-mix	Vendor A	Vendor B	Vendor C	Vendor D
CBG	Concentration (mg/ml)				
	0.2586	0.2500	0.2500	0.2507	0.2498
	C_{diff} vs. 11-mix				
		102.4%	99.7%	101.6%	101.6%

Discussion

Because analytical facilities often rely on standards from multiple vendors, consistency among reference material producers is paramount to producing valid results that are widely trusted. Although ISO 17034-accredited manufacturers implement a sound quality system and are deemed competent in meeting the highest quality standards for production of their reference materials, no study has been undertaken to compare the validity of reported concentrations across different vendors. Although there is known variability between CRMs as outlined above, here we show that within 10% variability, there is no difference in the reported concentration values of 11 single CRMs between vendors when compared against the concentration of a pre-made CRM mixture. This criterion considers the accepted variability between two batches of a CRM as well as any other variability introduced in the sample preparation prior to analytical testing. This demonstrates support for the rigorous stability testing that all CRMs, including single components and pre-made CRM mixtures, undergo to guarantee each component maintains its certified concentration by the specified expiration date. These CRMs from separate manufacturers appear interchangeable, allowing for more flexibility during routine lab testing.

By confirming that CRMs from separate ISO 17034-accredited reference material producers are exchangeable, analytical facilities can be confident in their results even when selecting reference materials from different ISO-accredited manufacturers. We demonstrate that adhering to the ISO 17034 standard enables reciprocation among reference material manufacturers, allowing the end user to obtain valid and reliable results.

Our data also verifies that a pre-made CRM mixture is as accurate as a mixture prepared in house from a set of single CRM components. In a previous study, we demonstrated that pre-made, multi-component CRM mixtures improve quantitation accuracy by avoiding some of the preparation errors that can occur while preparing a stock mixture from single CRMs.[1,2]

If you would like any more information on this data or would like to use Cayman CRMs in your lab, contact us at sales@caymanchem.com.

Find Cayman's phytocannabinoid 3-, 4-, 5-, 6-, 10-, and 11-component mixtures at www.caymanchem.com/phytomixture.

Cayman's 11-component phytocannabinoid mixture is also available from Shimadzu at www.ssi.shimadzu.com/11phytomix and is an integral part of their Cannabis Analyzer for Potency and Hemp Analyzer for Potency platforms.

References

1. Miller, M.G., Goodwin, S.K., and Franckowski, R.E. CRM mixtures improve quantitation accuracy. *Application Note, Cayman Chemical* (2019).
2. Miller, M., Goodwin, S., and Franckowski, R. Analytical data confidence is highest with commercially prepared CRM mixtures. *Cannabis Science and Technology* **2(4)**, 82-83 (2019).
3. DeSilva, B., Smith, W., Weiner, R.M., *et al.* Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. *Pharm. Res.* **20(11)**, 1885-1900 (2003).

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