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Infinite Chemical Analysis Labs Public Comments on DCC's potency standardized method ("Determination of Cannabinoids Concentration by HPLC for Dried Flower, including Pre-Rolls")

ICAL comments to specific sections in the method are provided in bold below.

Calibration standard solutions:
Prepare 0.5, 2, 5, 10, 20, 50 and 100 ppm calibration standard solutions as follows:

The current method's selection of calibration points is acceptable for the dynamic range chosen, but If we add more points to the curve there is no reason to achieve these exact values for standards. Calibration standard concentrations should be determined by the number of calibration points required and the required dynamic range. They should be reasonably spaced out based on these two factors. We would suggest using a fraction system of decreasing concentrations. For example: Std1 1xC for 100 ppm, Std2 ½ x C for 50 ppm, Std3 ⅕ x C for 20 ppm, Std4 1/10 x C for 10 ppm, Std5 1/20 x C for 5 ppm, Std6 1/50 x C for 2 ppm, and Std7 1/200 x C for 0.5 ppm. The guideline could be that all labs must achieve a calibration curve at a minimum range of 0.5 - 100 ppm, but the dynamic range can be extended without incurring additional cost and time. For example if a high calibration point of 300 ppm is desired, then Std2 = 150 ppm, Std3 = 60 ppm, Std4 = 30 ppm, Std5 = 15 ppm, Std6 = 6 ppm, Std7 = 1.5 ppm and Std8 ≤ 0.5 ppm.

From the homogenized composite sample, weigh 200 mg appropriate amount of sample, indicated below, that corresponds to the sample type into a labeled 50 mL centrifuge tube and record the weight

Our lab strongly disagrees with the use of only 200mg of sample for flower. Collecting a representative sample from the homogenized sample is not as easy as one might think. The smaller the sample size, the larger the variance in the analysis. The reason for this is the fact that keif is a smaller particle size than the plant material itself. The larger the sample size we can use from the homogenized sample, the more accurate and precise the results will represent the whole sampled batch. Infinite CAL has done these experiments in the past and believe that at minimum you need 0.5g for flower and 1.0 g for trim. We recommend that either the minimum sample size is increased or the language needs to be more explicit to state that 200mg is the minimum quantity required (i.e. weigh no less than 200 mg…).

Furthermore the method needs to be modified allowing a sample amount range. In example if 200mg is kept it should read 200mg+/- 10%. The exact mass should be used in the calculations for cannabinoid concentration.

Sample Extraction
1. Add 40 mL extraction solvent to the 50 mLl centrifuge tube with the sample.
For plant material, Use acetonitrile/methanol (80:20 Volume:Volume) as
extraction solvent.

The 80:20 ACN/MeOH mixture is not necessary for the efficient extraction of cannabinoids from plant material. The cost of ACN is much higher than MeOH. We have run hundreds of side by side extractions with many different solvent combinations and shown that MeOH extraction is equivalent or better at extracting from flower material. Our suggestion is to allow for other extraction solvents to be allowed to be used. Table 1 shows cannabinoid percentages for a flower sample from a side-by-side comparison of ICAL's analytical method, in which we use only MeOH as the extraction solvent, to the standardized method provided by the DCC, which requires 80:20 ACN:MeOH. Cannabinoid percentages are nearly equivalent with the relative percent difference between analytes <5%; additionally, the filtrate being analyzed would allow for lower LOQ in the DCC method.

Comparison of ICAL method and DCC method applied to a cryo ground flower sample			
Method	**ICAL Method**	**DCC Method**	**Relative % Difference**
Solvent	**MeOH**	**ACN:MeOH (80:20)**	
Overall Dilution (mL/g)	**850**	**4000**	
CBC (%)	0.0864	ND	N/A
CBD (%)	0.0080	ND	N/A
CBDA (%)	0.0650	ND	N/A
CBDV (%)	ND	ND	N/A
CBG (%)	0.2095	0.2027	3.30
CBGA (%)	1.0662	1.0286	3.60
CBN (%)	0.0115	ND	N/A

d8-THC (%)	ND	ND	N/A
d9-THC (%)	2.6666	2.6269	1.50
THCA (%)	21.9585	22.4095	2.03
THCV (%)	ND	ND	N/A
Total THC (%)	21.9242	22.2801	1.61

Table 1. Comparison of potency percentages for a flower sample from ICAL's potency method and DCC's standardized potency method diluted sample and the filtrate was not considered.

Extract in a sonicating bath for at least 30 minutes with ice in the water bath.

In our experience, the ice water bath is not needed. Extraction efficiencies are not significantly affected whether an ice water bath is used or not (see Table 1; ICAL's method for flower prep uses no sonication step at all, only vortexing for 15-20 min).If the concern with warmer conditions is the potential for decarboxylation of THCA, we do not observe any significant difference in amount of THCA or THCA/THC ratios using our method (which does not incorporate ice) and the DCC standardized method.

Centrifuge to 3900 rpm for 15 minutes.

We recommend that the procedure permit speeds greater than 3900 rpm (e.g.,"Centrifuge to 3900 rpm or greater); but if using a higher speed, the amount of time can be decreased as well."

Wavelength for detection:

Forcing a lab to use a wavelength of 220 nm limits the dynamic range of the calibration curve and LODs/LOQs. We suggest allowing for other wavelengths to be used if it can be verified.

Equilibrate the HPLC system with the mobile phases for at least 30 minutes.

30 minutes is an arbitrary length of time for equilibration. Equilibration time is dependent on flow rate and column size. It may be better to define this step based on column volumes rather than time.

2. A Laboratory Control Sample (LCS) is a blank matrix to which known concentrations of each of the target method analytes is added. The LCS is analyzed in the same manner as the representative sample. Methyl Cellulose powder is used as a blank matrix for this method and a mixture of 9 cannabinoids (CRMstandards) at a known amount is spiked into the blank matrix. Recovery of the LCS must be 70-130% of the spiked amount.

The 70-130% recovery range is far too large and should be reconsidered. The range is acceptable for lower level spikes but for high level spikes this is far too large of a range. Our suggestion is to base acceptance criteria on several analyte concentration ranges (i.e., low level, mid level, high level) where higher levels have lower variance and lower levels have higher variance.

The cost of doing the spikes in this manner (pre-dilution) is too costly considering the amount of standard that needs to be used; in order to still obtain necessary information on a spiked matrix, an alternative could be to only spike on selected analytes. I would suggest using D9-THC,THCA,CBD,CBG there is no need to spike all analytes. Another alternative to a single LCS or an LCS and PDS is to do an internal standard spike on all samples that would be more cost effective. The internal standard would not have to be used for inclusion in the calibration curve and for quantitation of analytes, simply to have a compound in each prepared sample solution that can have its recovery checked. Potential compounds for internal standards could be ibuprofen or benzoic acid. The advantage would be that it allows a check on every sample instead of a different matrix spike.

3. A Sample DuplicateLaboratory Replicate Sample (LRS) measures the precision of the analytical process. Duplicate analysis involves a replicate sample, sub-sampled in the laboratory. Method precision is documented and controlled based on the relative percent difference (RPD). The RPD must meet the acceptance criteria of RPD ≤30% as required by California Code of Regulations, title 4, section 15730.

The range for this acceptance criteria is far too large and should be reconsidered. The range is acceptable for lower level analytes but far too large for high level analytes. Our suggestion is to base acceptance criteria on several analyte concentration ranges (i.e., low level, mid level, high level) where higher levels have lower variance and lower levels have higher variance. From run to run you should see less then 5% RPD on the major cannabinoids.

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More Broad Points:

Double injection- **Currently the filtrate is required to be injected. If the dilution can achieve the required LOQ and LOD by the method there is no need to do so. This puts a financial burden on the laboratory. Not only is an additional filter, vial, and cap used, but the analytical column and instrument is being abused with such high concentrations. Columns and instruments will need to be changed or repaired more often.**

Currently standards used in this method require 1000 ug/mL concentration. **This should be modified to allow for any concentration that can be used to make the required calibration curve, ICV, or spike solution. Some cannabinoids are not made at this level because of stability issues; a good resource to ask is the standard manufacturer themselves. Furthermore finding two sources of standard can be difficult for additional cannabinoids.**

Should allow two curves, separate acids and non-acids (for stability's sake)-
DCC should allow the labs to prepare two separate calibration curves, separating the acids and the non-acids. ICAL has found that there is a significant difference in the stability of the acid standards when they are mixed with the non-acid standards, and mixing the two would require preparation of the calibration standards much more frequently and incur significant extra costs. Additionally, the DCC validated a method for only 9 analytes, but as labs begin validating additional analytes for this method, it will not be possible to mix together 11+ analytes at an initial concentration of 1000ug/mL and still hit the required concentration of 100ppm for standard 1 in the calibration curve.

Scientific advisory group for method modifications - **A comment session is not appropriate for method modifications. I would suggest creating a scientific advisory group to go over with the DCC science team and people that created or are modifying the method. A biweekly or monthly meeting would be the most beneficial to the DCC and appropriate to share ideas and discuss topics. The reason to allow for multiple meetings in participants can test theories in between the meeting and share findings. Current labs in CA can apply to participate in the group and provide feedback and have a scientific discussion about the method and improve it. The DCC science team would have the chance to learn and hear from scientists that have been doing this for years and potentially have more experience in this method. The stakeholders in labs are the best resources for this type of group and would donate their time to improve this analytical method. This would have**

no cost to the DCC and this is currently done in many states, for example Michigan. Many other regulatory bodies use similar methods to this with stakeholders meeting and improving or writing methods: For example, whenever the EPA wishes to introduce a new regulatory method, the draft that is released is not intended to essentially be the final result with minor language clarifications. The input by stakeholder labs is extensive, the EPA will usually request for several rounds by one or multiple labs to validate and provide feedback to the EPA before finalizing the method. This usually results in dramatic changes to the method before release.