Chemic Laboratories, Inc.
480 Neponset Street - Bldg. 7, Canton, MA 02021
Phone #: (781) 821-5600 facsimile #: (781) 821-5651

November 5, 2008

Anand K. Parekh, MD MPH
Office of Public Health & Science
U.S. Department of Health & Human Services
Anand.Parekh@hhs.gov

Dear Dr Parekh:

As per your request of June 18th please find the following response via email. Chemic Laboratories appreciates the reviewers' time and consideration surrounding the provided protocol. In order to provide clarification each point of interest has been specifically reviewed and detailed responses provided. Following your review of the provided responses please do not hesitate to contact me with any further questions or comments. Chemic Laboratories looks forward to your favorable responses.

Best regards,

Joseph St.Laurent
President, CSO

Chemic Laboratories, Inc.
480 Neponset Street - Bldg. 7, Canton, MA 02021
Phone #: (781) 821-5600 facsimile #: (781) 821-5651

November 5, 2008

1. THCA: the definition of THCA is not clear. Do the authors refer to the precursor to THC in plants that produce THC upon heating, or to 11-nor-Δ9-9-carboxy-THC, the metabolic product of THC? The author's state that the concentration of this compound will be determined, but the source of THCA is not provided and no further information on its detection, quantification, or results are provided? In addition, the physiochemical characteristics of this compound are quite different from the other cannabinoids and an appropriate internal standard should be included for quantification. It is quite possible that THCA in Cannabis Sativa products could differentially contribute to the amount of measurable THC in the three methods of analysis- Soxhlet extraction, vaporization and combustion. It is not known whether the THCA will decarboxylate at vaporization temperatures or whether it might decarboxylate during Soxhlet extraction (doubtful). This could introduce variability into the results. Has the THCA content been determined prior to testing? In general, NIDA does determine the THCA content of its cannabis' batches. This assumes THCA refers to the precursor acids.

THCA in the presented protocol refers to 11-nor-Δ9-9-carboxy-THC. The concentration will be determined by external standardization using a reference standard obtained from commercial sources (as applicable).

It is anticipated that the decarboxilation will occur during combustion and not using the various extraction procedures. It is the difference in isolation techniques that the authors are interested in.

2. Why would external standardization be necessary? Internal standardization is the preferred method of analysis. Would it not be feasible to fortify the Cannabis sativa plant material with internal standard rather than fortifying the methanolic solutions to account for losses during heating, absorption on the volcano parts or during combustion? Why is only one deuterated cannabinoid used as an internal standard? The committee believes that deuterated CBD and perhaps deuterated CBN may be available to improve quantification of these analytes.

It has been determined in previous studies that external standardization provides the necessary precision and accuracy to accurately determine the concentration of the cannabinoids isolated during the extraction and vaporization process. Although the CBD and CBN deuterated standards may be available, it has been determined in previous experimental investigations that the instrumental parameters described in the presented protocol support the relative response factors of the three analytes (i.e., CBD, CBN and THC) being equivalent, and therefore can estimate that the relative response factors of the deuterated analogs would be equivalent also.

3. Why is LCMS rather than LCMSMS utilized for identification of the cannabinoids? Why is only TIC used rather than single ion monitoring? How many ions are monitored for each cannabinoid (minimum three with two ion ratios gold standard analysis) and two minimum for each internal standard (one ion ratio)? Please submit the method validation criteria for the LCMS method showing sensitivity, specificity, accuracy or bias, imprecision, matrix effect evaluation, recovery (if performed), interferences, linearity, carryover evaluation and/or other criteria.

LC-DAD-MS is being used as the technique has been qualified in previous experiments to provide the necessary measure of precision and accuracy. DAD is being used for quantitation while TIC-MS is being used for identification. At a minimum three to five ions are used for identification and confirmation

4. What is known about pyrolytic conversion of the target cannabinoids by the volcano vaporizer and the combustion method?

Currently it has been determined that limited pyrolitic conversion occurs at the temperature prescribed within the protocol provided.

Chemic Laboratories, Inc.
480 Neponset Street - Bldg. 7, Canton, MA 02021
Phone #: (781) 821-5600 facsimile #: (781) 821-5651

5. Page 4 Tetrahydrocannabinol, cannabinol, cannabidiol and Tetrahydrocannabinol-acid are misspelled.

The finalized protocol will be amended to accommodate any misspelling.

6. Throughout the protocol data are used in the singular, data are always plural.

The finalized protocol will be amended to accommodate any grammatical issues. Please note where ever the term "Data" is used it is meant to indicate multiple sets of information. In the event a single informational point of interest is being made reference to the term "Datum" is used.

7. P 7 Why is the weight of the glass filter included in the total weight of the marijuana? Has potential absorption of cannabinoids to the balloon, reservoir or other parts of the volcano been determined?

Total lose of cannabinoids have been determined to be minimum in all the components with the exception of the glass fiber filter. It is for this reason that the tare and gross weight data are collected for all the samples tested.

8. P10 Acetonitrile is misspelled.

See comment #5

9. P10 evaluation criteria are inadequate.

Please note that all evaluation criteria established are in line with USP Category II validations and ICH criteria.

a. What are retention time criteria?

RT criteria is typically +/- 5%

b. What are appropriate chromatography criteria including peak shape, peak resolution, S/N?

Again USP criteria establishes peak resolution as > 2 and N/N >1:10 with >95%recovery

c. What criteria are in place for variability between the four calibrator injections within a single run?

Again variability in measured according to USP and ICH criteria. Variability between the analytes and multiple injections has been established as <5%

d. How many samples are included in each batch with the four calibration curves?

It is typical to include between 15 and 20 samples prior to assaying continuing calibration standard.

e. Are the concentrations of each calibrator individually determined against the entire curve?

Absolutely, as well as an acceptance criteria has been established in line with USP and ICH criteria.

Chemic Laboratories, Inc.
480 Neponset Street - Bldg. 7, Canton, MA 02021
Phone #: (781) 821-5600 facsimile #: (781) 821-5651

f. What is the acceptable concentration range? Are there procedures for eliminating specific calibrators if they do not meet the criteria?

Although it is not anticipated that calibrators will be dropped using statistical criteria such as Chauvenets criteria can allow for the removal of test standards. How many calibrators can be dropped from the curve? This has not been established at this time

g. Is there drift across a single batch with such a long run time (60 min)? Are calibrators prepared at the same time as samples?

Again continuing calibration accommodates any drift.

h. Where are quality control samples? Are quality controls (preferably 3 across the linear dynamic range of the curve) prepared independently and assessed at the beginning and end of the batch or more frequently?

Yes, QC samples are prepared at each of three concentrations and assayed across the samples set.

i. How were matrix effects evaluated? How were other endogenous compounds within the Cannabis sativa evaluated to assure lack of interference? Especially from other natural cannabinoids? Were cannabigerol, cannabichromene for example, evaluated for interference with target compounds?

All compounds are assayed with suitable resolution using HPLC-DAD-MS ensured quantitation and identity (i.e., specificity)

j. Although the compounds of interest appear to be present at concentrations well above the lower limit of linearity, what was the limit of quantification? Or was the lowest calibrator used for this purpose?

At this time the lower concentration from the standard curve is empirically determined as the limit of quantitation.

10. P11 Reference for Marijuana and Medicine was not included.

11. Was the valve, mouthpiece and/or filling chamber changed between samples? Is it changed routinely between subjects?

The valve and mouthpiece and filling chamber is not anticipated to be changed between test samples.

12. P 25. The common oven bag that is referenced is used for what application? Moisture content only or for delivery of cannabinoids.

The plastic oven bag is the means of collection of the vaporized THC and other cannabinoids

13. Why are no data presented for THCA with the other analytes?

THCA was added at the request of a previous reviewer; data is not available at this time

14. P32 the protocol indicates that all peaks with an area greater than the lowest calibrator area will be quantified, but it appears that uncalibrated peaks are not collected according to the instrument set up? Also, why is the origin included in the linearity? It is a bad practice to force the calibration line through the origin. However, the data did not appear to go through zero? What was done and what is the justification for this choice?

Chemic Laboratories, Inc.
480 Neponset Street - Bldg. 7, Canton, MA 02021
Phone #: (781) 821-5600 facsimile #: (781) 821-5651

November 5, 2008

The reviewer is correct all values greater than the lowest calibrator will be quantitated, as well as uncalibrated peaks will be collected for further evaluation at a future date. The origin is not being forced although the data at the null concentration is collected and placed into the curve to ensure any negative bias will be recognized during testing.

15. What is the internal standard for the PNA analysis? Was internal or external standardization used? Why not internal standardization if it was not the method used?

External standardization is being used however deuterated PNA standards are utilized as well.

16. P 40 Why was the Fragmentor ramp disabled? Why were multiple ions not monitored and ion ratios determined to specifically identify compounds?

It was determined in earlier experiments that that the analytes ionize with relatively equivalent fragmentation.