Quantitative determination of terpenes in Hemp and Hemp products by Gas Chromatograph using a Flame Ionization Detector or Mass Spectrum Detector

1.0 PURPOSE

1.1 This SOP provides documented procedures and requirements to guide the analysts in preparation, analysis, and quantification of major terpenes present in Hemp material.

2.0 THEORY AND PRINCIPLES

2.1 This is a GC FID or GC-MS method applicable to the determination of terpenes in different matrices of hemp/cannabis material using a Gas Chromatograph utilizing a Flame Ionization Detector or Mass Spectrum Detector. Terpenes are naturally occurring compounds found in the Hemp sativa plant. Terpenes are hydrophobic and are highly soluble in organic solvents. This method uses a liquid-liquid extraction with an organic solvent (methanol) to extract terpenes from hemp and hemp products. This is gas chromatography with separation via a capillary column at specific times for detection by a flame ionization detector or Mass Spectrum Detector. The linear relationship between terpene concentrations in relation to area peak height can determine the total percent terpenes in hemp and hemp products.

2.2 Analytes include (but not limited too):

<table>
<thead>
<tr>
<th>Method Analytes</th>
<th>CAS Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-Pinene</td>
<td>80-56-8</td>
</tr>
<tr>
<td>Camphene</td>
<td>79-92-5</td>
</tr>
<tr>
<td>Beta-Pinene</td>
<td>18172-67-3</td>
</tr>
<tr>
<td>Myrcene</td>
<td>125-35-3</td>
</tr>
<tr>
<td>d-3-Carene</td>
<td>13466-78-9</td>
</tr>
<tr>
<td>a-Terpinene</td>
<td>99-86-5</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>99-87-6</td>
</tr>
<tr>
<td>Limonene</td>
<td>5989-27-5</td>
</tr>
<tr>
<td>Eucalyptol</td>
<td>470-82-6</td>
</tr>
<tr>
<td>Ocimene</td>
<td>13877-67-3</td>
</tr>
<tr>
<td>g-Terpine</td>
<td>99-85-4</td>
</tr>
<tr>
<td>Linalool</td>
<td>78-70-6</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>586-62-9</td>
</tr>
<tr>
<td>Isopulegol</td>
<td>89-79-2</td>
</tr>
<tr>
<td>Geraniol</td>
<td>106-24-1</td>
</tr>
<tr>
<td>b-Carophyllene</td>
<td>87-44-5</td>
</tr>
<tr>
<td>a-Humulene</td>
<td>6753-98-6</td>
</tr>
<tr>
<td>c-Nerolidol</td>
<td>3790-78-1</td>
</tr>
<tr>
<td>t-Nerolidol</td>
<td>40716-66-3</td>
</tr>
<tr>
<td>Carophyllene Oxide</td>
<td>1139-30-6</td>
</tr>
<tr>
<td>Guaiol</td>
<td>489-86-1</td>
</tr>
<tr>
<td>a-Bisabolol</td>
<td>23089-26-1</td>
</tr>
</tbody>
</table>
3.0 DEFINITIONS

3.1 Batch – A group of samples analyzed on one calendar day.

3.2 Method Blank (MB) – An aliquot of blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents. The MB is used to determine if method analytes or other interferences are present in the laboratory equipment, the reagents, or the apparatus.

3.3 Laboratory Control Sample (LCS) – An aliquot of blank matrix to which known quantities of representative method analytes are added in the laboratory. The LCS is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.4 Stock Standard Solution – A concentrated solution containing a single certified standard that is a method analyte or a concentrated solution of multiple analytes either prepared in the laboratory or purchased as a certified stock standard solutions. Stock standard solutions are prepared in HPLC grade methanol.

3.5 Internal Standard (IS) – A known concentration of a compound (Tridecane) added to every sample and standard analyzed.

3.6 Working Calibration Standards – Different concentrations of analytes prepared in methanol used to calibrate the instrument for an area response with the respect to analyte concentration.

3.7 Continued Calibration Verification (CCV) – An analytical standard prepared from the same source as the calibration standards that is analyzed periodically prior to, during, and/or after analysis of samples to verify the continued accuracy of an instrument calibration.

4.0 SUMMARY OF METHOD

4.1 All Samples are grinded to a fine powder and a homogenous sample of the material is weighed for analysis. A known volume of HPLC grade methanol is added to the sample, shaken then placed into the ultrasonic bath for 10 minutes. The sample extract is filtered through PTFE filter (if needed) then analyzed. The sample is introduced through gas chromatography with a gradient oven ramp through a Phase 5 capillary column with analyte detection by flame or mass spectrum detection. The percent or concentration of terpenes is determined by the concentrations for each analyte. Terpenes are reported in total percent or mg/g for flower, concentrates, and tincture samples. Edibles are reported mg/dose potency.

4.2 Quality control samples (MB, LCS) are treated like samples and analyzed along with the samples to determine the analysis is in control.
5.0 RESPONSIBILITIES

5.1 It is the responsibility of the cultivators or manufactures to submit samples as they see fit. Until a sample is received by Dawn Analytical it is the responsibility of the producer.

5.2 It is the responsibility of the analyst performing this analysis to read and be familiar with the procedures and requirements of this SOP prior to reporting sample results. It is also the responsibility of the analyst to review and adhere to all laboratory safety policies applicable to this procedure.

6.0 SAFETY

6.1 Safety and personal protective gear required.

6.2 Some of the reagents and standards used in this method are hazardous. Before using this method, individuals should read all related material safety data sheets (MSDS).

6.3 Dispose of waste into proper containers.

7.0 REQUIREMENTS (Equipment, Apparatus, Materials)

7.1 Equipment

7.1.1 Gas Chromatograph with FID and MSD
7.1.2 Autosampler which includes Tray and Tower
7.1.3 GC Phase 5 Capillary Column
7.1.4 Analytical balance
7.1.5 Sonicator

7.2 Solvents

7.2.1 Methanol

7.3 Materials

7.3.1 Terpene Standards
7.3.2 Tridecane Standard (Internal Standard)
7.3.3 Vial for extraction
7.3.4 Autosampler vials
7.3.5 Microsyringes
7.3.6 Serological transfer Pipets

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 All samples are stored in the dark to prevent degradation of THC from light.
8.2 Sample size required is 1.0g of sample or 2 full edibles.
8.3 Sample hold time is 7 days. All samples must be analyzed within 7 days of collection.
8.4 Sample turnaround time (TAT) is 36 hours, unless otherwise specified on Work order.
8.5 All unused samples after analysis are stored in the dark for 365 days.
8.6 Sample used waste is disposed of in the proper waste drum.
8.7 Analysis waste is disposed of in the proper waste drum.

9.0 PROCEDURE

9.1 Calibration Curve Preparation
9.1.1 Prepare or purchase Stock Standard Solutions of the individual cannabinoids.
9.1.2 Prepare 3-5 calibration points to be analyzed on the GC-FID or GC-MS system. All documentation as their preparation is noted in the Standards Log.

9.2 Internal Standard Preparation
9.2.1 Prepare an Internal Standard by placing a known weight of Tridecane in a vial with a known quantity of Acetone/Hexane/Methanol solvent mixture.
9.2.2 Calculate the ug/ml concentration of that standard.
9.2.3 Prepare a working internal standard by preparing a 150ug/ml standard. Prepare at least 10-20mls of the working IS to allow for lengthy use in time.

9.3 Prep Batch Sheet Preparation
9.3.1 Complete a prep batch sheet for all samples to be analyzed. This will include one MB and one LCS for each day samples are extracted. Each MB and LCS is a blank vial with 4mls Methanol. The LCS in addition has the 100mg of the LCS Sample added.
9.3.2 Aliquot 0.5mls of both the MB and LCS to a 2ml autosampler vial and add 5ul of IS.

9.4 Samples
9.4.1 Grind the whole sample into a fine powder.
9.4.2 Weigh out 100mg of sample into tarred 4ml vial and record weight on to Batch Prep sheet by sample ID. Making sure to label each vial tube with sample id.
9.4.3 Add 4ml of MEOH to each vial, shake, and sonicate for 10 minutes.
9.4.4 Filter (if needed) sample through PTFE filter.
9.4.5 Aliquot 0.5ml of filtered extract into 2ml autosampler vial. Add 5ul of IS to autosampler vial.
9.4.6 Proceed onto section 10 instrument set up.

10.0 INSTRUMENT SET-UP
10.1 Turn on the GC before turning on computer software HP Chemstation.
10.2 Load method TERPENE.M and turn on air generator. Allow the air to build pressure and then turn on Air and Hydrogen to FID. Ignite FID. Let system stabilize and equilibrate.
10.3 Open sequence parameters, add analyst name and save as date to be analyzed. Load sequence table adding either a new calibration curve or CCVs to verify calibration curve has
held from previous day of analysis. Next add MB, LCS, and samples; save sequence as date of analysis.

10.4 A Continuing Calibration Verification (CCV) needs to be analyzed every 10 samples. A CCV is the any of the following calibration points which are analyzed every 10 samples, bracketing the samples throughout the analytical run to ensure system suitability.

10.5 Recommended Instrument operating procedures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Type</td>
<td>Phase 5 Column</td>
</tr>
<tr>
<td>Column Temperature</td>
<td>50°C</td>
</tr>
<tr>
<td>Oven Ramp Time</td>
<td>Int. Temp: 50 degrees Time: 1 min</td>
</tr>
<tr>
<td></td>
<td>6 degrees per min to 80 degrees</td>
</tr>
<tr>
<td></td>
<td>20 degrees per min to 250 degrees</td>
</tr>
<tr>
<td></td>
<td>Final Time: 1 min</td>
</tr>
<tr>
<td>Flow</td>
<td>5.0 ml/min H2</td>
</tr>
<tr>
<td>Detection</td>
<td>FID or MSD</td>
</tr>
<tr>
<td>Injection volume</td>
<td>1 µl</td>
</tr>
<tr>
<td>Injection Port and FID Temp</td>
<td>250 degrees</td>
</tr>
</tbody>
</table>

11.0 ANALYSIS

11.1 Prepare GC as described in section 10.

11.2 Positive identification of single component analyte is made by comparison of the retention times (RT) of the target analytes in the standards and quality control samples to the component peaks exhibited by the sample on the FID. Positive identification of analytes is made by comparison of the retention times of the analytes and absorption and excitation of the analytes.

11.3 The retention time window for each analyte is defined as the sum of the area of the identified analyte peak integrated “baseline to baseline” as opposed to valley to valley. Baseline to baseline is defined as a flat baseline drawn that includes all the responses within the retention time window of each different analyte within relation to baseline of stabilized instrument.

11.4 The experience of the analyst with the method and equipment being used weighs heavily in the application of retention time windows and final interpretation of chromatograms.

12.0 INSTRUMENT SHUT-DOWN

12.1 Upon completion of analyses, the GC can be left in idle state. The FID gases can be turned off to conserve gas. The Air generator can be turned off.
13.0 PREVENTATIVE MAINTAINANCE AND REPAIR
13.1 Instrument waste container is emptied daily into the proper waste drum for disposal.
13.2 All preventative maintenance or repairs are logged in the instrument maintenance log.
13.3 Routine maintenance will need to be conducted on a regular basis. Routine maintenance includes, replacing septa, liner, old seal, column, jet, or any other consumable items.

14.0 QUALITY CONTROL
14.1 Calibration and Standardization
14.1.1 Preparation of Calibration Standards it is recommended that a linear curve be utilized for better quantitation results. 3-5 calibration points are prepared using standards which consists of a mix of all analytes of interest. The lowest level standard should represent analyte concentrations close to the required reporting limits. The remaining standards should bracket the analyte concentrations expected in the sample extracts and/or define the working range of the detector.
14.1.2 Calibration Standards must be analyzed under the same operating conditions that the samples will be analyzed.
14.1.3 The calibration curve for each compound being analyzed must meet a requirement of coefficient $r^2 \geq 0.99$ for curve-fit.
14.1.4 During sample analysis following initial calibration the calibration curve must be verified at a minimum frequency of every 12 hours or after the analysis of every 10 samples, whichever is more often, by the measurement of a mid-level CCV standard. The calibration curve must also be verified by a CCV at the end of the analysis sequence, or each new day of analysis. If the response is within 20%, the standard verifies the current curve. If the standard fails a new calibration curve will need to be analyzed. Samples analyzed in association with the failed CCV must be reanalyzed. However, in the event that an analyte of interest in the continuing calibration verification exhibits a greater than expected response (i.e. high bias) and the analyte is not detected in an associated sample, that sample need not be reanalyzed for that analyte.

14.2 Method Detection Limits (MDL) study
14.2.1 The MDL study demonstrates the laboratory’s ability to generate acceptable data with accuracy and precision, and should be performed for each analyte and matrix prior to analyzing any samples, and then at least annually thereafter. An MDL is also required for each instrument prior to analyzing the analytes in this SOP for sample reporting.
14.2.2 Prepare at least seven replicates spiked at a level equal or lower than your level 1 standard of 12.5µl/ml. Calculate the average concentration (x) found and the standard deviation of the concentrations for each analyte. The % RSD should be <5.

14.3 **Third-party Blind Quality Control Samples (QCS)** – i.e. PT Samples-should be analyzed routinely to demonstrate the laboratory’s ability to generate accurate results.

14.3.1 Minimum quality control (QC) requirements are Initial Demonstration of Competency (IDC), annual Method Detection Limit studies (MDLs), analysis of method blanks (MB), laboratory control samples (LCS), and CCVs.

14.3.2 Third party Blind samples do not exist for Terpene analysis. When and if they do start by Proficiency Test providers Dawn Analytical will participate in those studies.

14.4 **Method Blank (MB)**

14.4.1 The MB is prepared and analyzed at a minimum frequency of 1 per batch or 1 each day that samples are prepared and analyzed. An aliquot of reagent water or other blank matrix is prepared exactly as samples. The MB is used to determine if method analytes or other interferences are present in the laboratory equipment. If interferences are present greater than the lower reporting limit, then the batch of samples and QC samples must be re-analyzed, after determining source of interference and completing a corrective action.

14.5 **Laboratory Control Spike and Laboratory Control Spike Duplicate (LCS)**

14.5.1 The LCS should be prepared and analyzed each day that a sample batch is prepared. It is extracted and analyzed exactly like a sample. Its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

14.5.2 Recoveries for target analytes in LCS must fall within the control limits of 70-130% or reanalysis is required of associated samples.

### 15.0 TRAINING

15.1 The analyst is responsible for reading and understanding the SOP and MSDS’s associated with any standards or solvents.

15.2 The analyst will observe the analysis being performed by an experienced analyst. Then the analyst will be observed by the experienced analyst preforming the analysis.

15.3 **Initial Demonstration of Capability (IDC)**

15.3.1 The IDC study is used primarily to preclude an analyst or laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining
some experience with it. An IDC is also required for each instrument analyzing the analytes in the SOP and when significant changes in instrumentation are made. An MDL may also satisfy this requirement.

15.3.2 Prepare and analyze at least three LCSs spiked with target analytes at a representative concentration for each compound. For each analyte, the recovery value for all 3 LCSs must fall within 20% from predicted response and RSD<5%.

15.4 Method Detection Limits (MDL) study. The MDL study demonstrates the laboratory’s ability to generate acceptable data with accuracy and precision, and should be performed for each analyte and matrix prior to analyzing any samples, and then at least annually thereafter. An MDL is also required for each instrument prior to analyzing the analytes in this SOP for sample reporting.

15.5 Continued annual training will require review of SOP, internal audit, IDC’s and MDL’s.

16.0 DATA ANALYSIS AND REPORTING

16.1 Calculation of Percent Recovery (%) for LCS. Report percent recovery for a LCS analyte as follows:

\[ \% = \frac{100(n)}{\text{Fortifying concentration}} \]

where (n) represents the analytical result of the LCS analyte.

16.2 Calculation of total Cannabinoid is determined by adding the Percent (%) for each cannabinoid as follows:

\[ \% = \frac{[\text{concentration}_{\text{ug/ml}}(\text{Volume ml})/1000]}{\text{Sample mass mg}} \]

Edible % = \[\frac{[\text{Concentration ug/ml}(\text{Volume ml})/\text{Sample mass mg}]}{1000}\]

16.3 Any results that are below the RL of 200 ug/g are reported as non-detect ND.

16.4 A QC Checklist is filled out by the analyst, which serves as a checklist of samples and QC samples with acceptable criteria.

16.5 Analyst shall print the Method information that the samples were analyzed with, along with the sequence ran, and calibration curve and plots. Each chromatogram is reviewed, printed and recorded onto the QC Checklist. A data package is created by placing the QC checklist with the method info, the sequence, calibration plots, QC samples and CCVs chromatograms. All client sample chromatograms are attached to the internal potency
report for the supervisory analyst to review, listing any qualifiers or discrepancies on the internal report. Corrective Action will be completed when any QC samples do not meet acceptance criteria.

16.6 The supervisory analyst will review data following the QA checklist to make sure all QC samples are within acceptable criteria limits, the calibration plots $r^2 \geq 0.99$, and check for grammatical errors. The data package is reviewed by the supervisory analyst and results are input into the final report sent to client via PDF.

16.7 LCS results are added to the control charts, making sure all results are within 2 sigma of the true value. If the LCS are greater than 3 sigma corrective action shall be completed.

16.8 For samples that have duplicates performed a %D of 20% shall be noted and the sample re-analyzed.

17.0 RECORD KEEPING

17.1 Final Data generated by the GC and reported are final results, data generated during the analysis procedure, including chromatograms, calibration data, and instrument setup documentation are stored electronically and backed up to an external hard drive periodically. A hard copy of raw data and reports are stored in for 5 years.

18.0 WASTE AND HAZARDOUS MATERIALS

18.1 All samples must be stored in an airtight container, labeled stored for 365 days in the dark.

18.2 All other wastes can be added to the waste drum.

19.0 ATTACHMENTS

19.1 Appendix #1 – Batch Prep sheet
19.2 Appendix #2 – Maintenance Log
19.3 Appendix #3 – QC frequency

20.0 APPROVAL WITH NAMES, TITLES, DATE AND SIGNATURE OF THOSE RESPONSIBLE FOR THE REVIEW AND APPROVAL OF THE ANALYTICAL TEST PROCEDURE

Approved By: ____________________________ Date: ____________

Mike Klasner
### Appendix 1

**Prep Batch Template**

#### HPLC/GC Prep Batch

<table>
<thead>
<tr>
<th>Date:</th>
<th>Analyst:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mass of Sample (mg)</th>
<th>Volume of MEOH (mL)</th>
<th>Volume of H2O (mL)</th>
<th>Total mass of edible (mg)</th>
<th>Internal Standard added to extract?</th>
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### Appendix 2

**Maintenance Log**

Instrument: Gas Chromatograph with Flame Ionization Detector, Autosampler which includes an injector and tray  
Condition: Good  
Put in use: 04/30/2016

<table>
<thead>
<tr>
<th>Date</th>
<th>Analyst</th>
<th>Maintenance Performed/Corrective Action</th>
<th>Comment on Damage or Malfunction</th>
<th>Part replaced</th>
<th>Back in Service?</th>
</tr>
</thead>
<tbody>
<tr>
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</table>
### Appendix 3

**QC Frequency checklist**

<table>
<thead>
<tr>
<th>Description</th>
<th>Frequency</th>
<th>Acceptance Criteria</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Demonstration of Capability (IDC)</td>
<td>Once per analyst prior to analyzing samples.</td>
<td>Four LCS’s Recovery ±20% from predicted response, %RSD &lt; 15</td>
<td>Repeat the IDC process.</td>
</tr>
<tr>
<td>Method Detection Limits</td>
<td>7 Replicates spiked at 1 to 5 times the reporting limits, completed annually.</td>
<td>Calculated MDL ≤ lower reporting limit for samples. %RSD &lt; 5.</td>
<td>Repeat the MDL process.</td>
</tr>
<tr>
<td>Initial Calibration (ICAL)</td>
<td>Prior to sample analysis if CCV does not meet criteria</td>
<td>% RSD ≤ 0.99 for each analyte</td>
<td>Re-analyze standards for curve</td>
</tr>
<tr>
<td>Continuing Calibration Verification (CCV)</td>
<td>Analyze every 10 samples</td>
<td>%D ≤ 20% for each analyte</td>
<td>Re-analyze standards for curve</td>
</tr>
<tr>
<td>Method Blank (MB)</td>
<td>Analyze daily prior to sample analysis</td>
<td>&lt; Reporting Limit for each analyte</td>
<td>Reanalyze once, if criteria still not met all samples must be re-extracted</td>
</tr>
<tr>
<td>Laboratory Control Sample (LCS)</td>
<td>Analyze daily prior to sample analysis</td>
<td>± 30 % from predicted response.</td>
<td>Reanalyze once, if criteria still not met all samples must be re-extracted</td>
</tr>
</tbody>
</table>