SOP No.: PT-METH-031		Page 1 of 8
Title: Determination of Delta-9-THC in Hemp by Gas Chromatography with Flame Ionization Detection (GC/FID)		
Revision: Original	Replaces: n/a	Effective: 09/23/2014

1. Purpose:

This standard operating procedure (SOP) describes the extraction of tetrahydrocannabinol (THC) from hemp vegetation followed by analysis using the Agilent 6890N Gas Chromatograph with a Flame Ionization Detector (GC/FID).

2. Scope, Responsibilities, and Authorities:

This SOP applies to staff members working in the Chemical Sciences and Pesticides Unit at the CDA-BCL. This SOP shall be used in conjunction with the SOPs listed in the reference section. Chemical Sciences and Pesticides Unit staff members shall adhere to the requirements specified in this SOP and report non-compliance to the Quality Assurance Officer (QAO) and/or the Laboratory Manager. The Laboratory Manager and QAO are authorized to request and review records.

3. Outline of Procedure:

6.1 Safety Precautions and General Requirements
6.2 Resources: Reference Materials, Chemicals, Reagents, and Equipment
6.3 Reagent Preparation
6.4 Standard, Spike, and Calibration Standard Preparation
6.5 Sample Preparation
6.6 Sample Analysis
6.7 Instrument Conditions
6.8 Quality Control
6.9 Detection Limits, Accuracy, Precision, and Measurement Uncertainty
6.10 Data Review and Reporting
6.11 Records
6.12 Sample Disposal and Clean-up

4. References, Related Procedures, and Forms:

External Documents

ED628 – Gas Chromatographic Determination of Tetrahydrocannabinol in Cannabis, Chemistry Section, Bureau of Drug Research Health Protection Branch, Health Canada, October 1992.
ED629 – Marijuana Potency Testing – Quick and Easy by GC or LC, Restek application note, 2012.
ED640 – Medical Marijuana Solvent Extraction Efficiency – Potency Determinations with GC-FID, 2011.

CDA-BCL SOPs and Manuals (current revision)

PT-INST-024 Operation and Maintenance of the Agilent 6890N GC/FID
PT-LBOP-014 Hemp Sample Preparation
SL-ADMIN-013 Training of Laboratory Personnel
SL-DATA-002 Data Collection, Review, Approval, Reporting, Storage, Protection, and Confidentiality
SL-QAQC-015 Analytical Method Quality Control
SL-QAQC-022 Method Validation and Verification

CDA-BCL Forms
PTM031A THC in Hemp Bench Sheet,(current revision)
PTM031B THC Potency (current revision)
SLL001I Intermediate Standard Preparation Log (current revision)
SLL001J Working Standard Preparation Log (current revision)

5. Definitions:

5.1 Cannabidiol (CBD) - one of at least 85 active cannabinoids identified in cannabis. It is a major phytocannabinoid, accounting for up to 40% of the plant's extract. CBD is considered to have a wider scope of medical applications than tetrahydrocannabinol.

5.2 Cannabinol (CBN) - a weak psychoactive cannabinoid found only in trace amounts in Cannabis sativa and Cannabis indica. It is mostly a metabolite of tetrahydrocannabinol.

5.3 Δ^9-tetrahydrocannibinol (THC) – cannabinoid that is the primary psychoactive constituent of marijuana and hemp products.

5.4 Matrix Control (MC) – Matrix matched standard that contains no analyte of interest.

5.5 Matrix Spike (MS) – Matrix matched standard fortified with a known concentration of THC.

5.6 Method Control (MEC) – a blank water sample to which all reagents are added in the same volumes or proportions as are used in the analysis of regular samples. The method control is carried through the complete sample preparation and analytical procedure. The method control is equivalent to a method blank.

5.7 Percent equivalent calibration standard – prepared to represent the response equivalent to that percentage of THC in the unknown sample based on the 0.20 mg sample weight.

6. Specific Procedures:

6.1 Safety Precautions and General Requirements.

6.1.1 Analysts that are not trained and authorized per SOP SL-ADMIN-013 shall not independently analyze customer samples by performing the procedures detailed in this SOP, including the operation and/or maintenance of the equipment and instrumentation utilized in this procedure.

6.1.2 Individuals using this procedure shall be familiar with the toxicity of all chemicals involved prior to use by reading the specific MSDS sheets. Appropriate personal protective equipment (PPE) shall be worn while performing work under this procedure.

6.1.3 The minimum quality control requirements listed in SOP SL-QAQC-015 shall be followed in this procedure.

6.1.4 Where possible, reference materials shall be purchased from a provider accredited to ISO Guide 34 in combination with ISO/IEC 17025.

6.2 Resources: Reference Materials, Chemicals, Reagents, and Equipment

Reference Materials

6.2.1 Cannabinoids standard, 1000-µg/ml ea CBD, CBN and THC, Restek or equivalent

Reagents

6.2.2 Methanol – Fischer Scientific, ACS grade or equivalent

Equipment

6.2.3 Gas chromatograph/flame ionization detector (GC/FID) – Agilent 6890N or equivalent

6.2.4 Analytical Balance

6.2.5 Graduated Cylinder, 50-ml, Class A

6.2.6 Pipettes, 20-µl to 200-µl

6.2.7 50-ml plastic centrifuge tubes

6.3 Reagent Preparation

6.3.1 No reagent preparation is necessary.

6.4 Standard, Spike, and Calibration Standard Preparation

6.4.1 Intermediate standard preparation shall be recorded on form SLL001I. Working standard preparation shall be recorded on form SLL001J.

6.4.2 Stock Standard Solution

6.4.2.1 1000-µg/ml Cannabinoids mixture in methanol, neat standard from Restek

6.4.3 Intermediate Standard Mixtures

6.4.3.1 No Intermediate Mixtures

6.4.4 Spike Standard Mixture

6.4.4.1 Spike 0.2g of matrix control material with 0.600 ml of stock standard.

6.4.5 Matrix Matched Calibration Standards

6.4.5.1 Levels are prepared to match expected responses at corresponding percentages of THC in the 200 mg sample. Example: 0.30% of a 0.200 g hemp sample is 0.60 mg or 600 µg. Extraction in 40 ml of methanol would result in a concentration of THC at 600 µg/40 ml which equals 15 µg/ml.

6.4.5.2 Calibration Levels: Prepare 6 calibration levels in control hemp matrix as follows:

Level	Standard (µg/ml)	Amount (ml)	Dilution (ml)	Conc. (µg/ml)	% THC
1	1000	0.100	1.0	100	2
2	1000	0.050	1.0	50	1
3	1000	0.025	1.0	25	0.5
4	100 (1)	0.150	1.0	15	0.3
5	50 (2)	0.100	1.0	5	0.1
6	25 (3)	0.100	1.0	2.5	0.05

6.5 Sample Preparation

6.5.1 Prepare the hemp samples per PT-LBOP-014.

6.5.2 Weigh 0.2 ± 0.05 g prepared hemp sample into a 50-ml centrifuge tube and record the weight on form PTM031A.

6.5.3 Add 40 ml methanol to the centrifuge tube. Cap and shake to ensure that the entire sample is wet.

6.5.4 Place on the Geno Grinder for 5 minutes at 500 rpm.

6.5.5 Allow the samples to settle for at least one hour or until the sample solution is clear.

6.5.6 Dispense a 1-ml aliquot of the sample solution into a 2-ml amber autosampler vial.

6.5.7 Samples may be stored in the pesticide sample refrigerator (CDA1010) until analysis is completed.

6.6 Sample Analysis

6.6.1 Samples shall be analyzed by GC/FID. Refer to PT-INST-024 for specific operational procedures.

6.7 Instrument Conditions

6.7.1 GC-FID instrument parameters:

Oven
Initial Temp: 200° C at 0 min
Ramp: 15° C/min to 300° C, hold 0 min

Front Inlet

Mode:	Split
Initial temp:	250° C
Pressure:	9.52 psi
Split ratio:	20:1
Split flow:	15.6 ml/min
Total flow:	19.1 ml/min
Gas Saver:	On
Saver flow:	20.0 ml/min
Saver time:	2.00 min
Gas type:	Helium

6.8 Quality Control

6.8.1 Sample sets shall contain one set of QC per each batch of 20 samples. Each set contains a method control, matrix control, matrix spike, and randomly chosen duplicate.

6.8.2 Sample values shall be within the method calibration range.

6.8.3 Duplicate samples should be within ±15% of each other.

6.8.4 The Method Control (Method Blank) should not have any detection greater than the method detection limit.

6.8.5 The Matrix Control (Matrix Blank) should not have any detection greater than the method detection limit.

6.8.6 Matrix Spike Criteria

6.8.6.1 The matrix spike shall be spiked at 0.1% THC.

6.8.6.2 The matrix spike recovery shall fall within the current limits established by control charted data trends.

6.8.6.3 Spike recoveries falling outside of the accepted range shall be evaluated and possibly re-injected. Continued failure of spike recoveries shall be investigated by the Work Unit Leader and a corrective action initiated in SharePoint.

6.8.7 Instrument Calibration

6.8.4.1 The calibration correlation coefficient (r^2) shall be $\geq$0.995.

6.8.4.2 It is acceptable for the initial instrument calibration to be used for analysis of samples if a continuing calibration standard is analyzed and within ±15% accuracy.

6.8.4.3 Calibration integrity shall be calculated for each sample set by analyzing a continuing calibration verification (CCV) standard. Results of calibration integrity shall be documented in the sample set report.

6.8.4.4 Continuing calibration standards shall be injected at the following intervals in the sample sequence; after calibration before the sample set, after every 20 samples, and at the end of the sample run.

6.9 Detection Limits, Accuracy, Precision, and Measurement Uncertainty

6.9.1 Detection limits were established per SOP SL-QAQC-022 and are 0.0671%.

6.9.2 Control Charting shall be performed and monitored per SOP SL-QAQC-015.

6.9.3 Accuracy and Precision is calculated according to SOP SL-QAQC-022 and is monitored through Control Charting per SOP SL-QAQC-015. Precision was verified with 7 replicates of matrix spiked at 0.3% THC and is 8.3. The sample standard deviation of 7 replicates was 0.021. The Measurement uncertainty (calculated as 2 x standard deviation) for matrix spike results is 0.043 and the %RSD is 8.07%.

6.9.4 Accuracy was measured as average relative percent error calculated as:

% Error = absolute value [(certified value – result)/certified value]*100%.

The average % error is 14.2%. Average percent recovery is 85.7%.

6.9.5 Potential sources of measurement uncertainty for this procedure include, but are not limited to:

- Quality of reference standards
- Preparation of stock, process and spike solutions
- Preparation of reagents
- Equipment variances
- Quality of chemicals and reagents
- Sample matrix
- Condition of samples upon receipt
- Extraction process
- Staff member performing analysis

6.10 Data Review and Reporting

6.10.1 Analysts shall review and summarize the raw data for each data set using form PTM031B.

6.10.2 Analysts shall provide completed summary reports and raw data to the Quality Assurance Unit (QAU) for review.

6.10.3 Data shall be evaluated for acceptability based on the Quality Control section of this SOP and SOP SL-QAQC-015.

6.10.4 The data shall be approved per SOP SL-DATA-002 prior to release to customer(s).

6.11 Records

6.11.1 Completed data packages are stored and archived according to the requirements listed in SOP SL-DATA-002.

6.11.2 Data shall be made available for internal and external audits, inspections, assessments, and reviews.

6.12 Sample Disposal and Clean-up

6.12.1 After the sample results and associated quality control have been electronically transmitted to the client, the sample extracts, analytical extracts and the raw samples shall be disposed of as follows:

6.12.1.1 Sample extracts : Non-chlorinated hazardous liquid waste

6.12.1.2 Analytical extracts : Non-chlorinated hazardous liquid waste

6.12.1.3 Raw Samples: place in trash receptacle if THC content is passing. Retain sample in secure storage if THC content fails.

7. Revisions:

NA – Original Version

8. Approvals:

Original Authors: Eric Petty and Daya Mitchell

Keith Wegner 09/23/2014

Approved By: Keith Wegner Date
Laboratory Manager, CDA-BCL

Ellen LaRiviere 09/23/2014

Approved By: Ellen LaRiviere Date
Quality Assurance Officer, CDA-BCL

COLORADO
Department of Agriculture
Inspection & Consumer Services Division

Title: THC in Hemp Bench Sheet Number: PTM031A Version: 01	Procedure No.: PT-METH-031 Revision: Original
Equipment/Instrument No.: CDA496, GC-FID #12	Page 1 of 2

Materials: **Date:** _____

Components	***Initials***	***Comments***
Cannabinoid Fortification:_____Exp:_____ Cannabinoids for Standards:_____Exp:_____ Methanol (ACS grade)_____Exp:_____ Pipettes:_____ Balance:_____	_____ _____ _____ _____	Investigations: Sample Numbers: Control Matrix Reference: Method Control: 40 ml methanol

Hemp Sample Preparation: **Date:** _____

Procedure	***Initials***	***Comments***
Remove Samples from storage.		
Prepare samples per PT-LBOP-014.		

Matrix Spike Preparation (MS): **Date:** _____

Procedure	***Initials***	***Comments***
Weigh 0.2 ± 0.05 g of control into a 50-ml centrifuge tube and record the weight in the Sample Weight Table.		
Add 200 µl of the Stock Standard Solution (0.10 % THC equivalent)		

Matrix Control Preparation (MC): **Date:** _____

Procedure	***Initials***	***Comments***
Weigh 0.2 ± 0.05 g of control into a 50-ml centrifuge tube and record the weight in the Sample Weight Table.		

Method Control Preparation (MEC): **Date:** _____

Procedure	***Initials***	***Comments***
Add 40 ml methanol to a 50-ml centrifuge tube and record in the Sample Weight Table.		

Sample Extraction: **Date:** _____

Procedure	***Initials***	***Comments***
Weigh 0.2 ±0.05 g of each sample into a 50-ml centrifuge tube and record the weight in the Sample Weight Table.		
Add 40 ml of Methanol to each tube. Cap and shake.		
Place samples on the Geno Grinder for 5 minutes @ 500 rpm.		
Remove samples and allow them to settle for at least 1 hour or until the extraction solution is clear.		
Dispense 1-ml of the extraction solution into a 2-ml amber autosampler vial.		

Sample Weight Table: **Date:** _____

	Sample Number	***Weight (g)***	***Initials***	***Comments***
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
Dup				
MC				
MS				
MEC				

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Title: THC Potency Data Analysis Worksheet	Related Procedure No.: PT-METH-031
Number: PTM031B	Revision: Original
Version: 01	
Equipment/Instrument No.: CDA496, GC-FID #12	Page 1 of 1

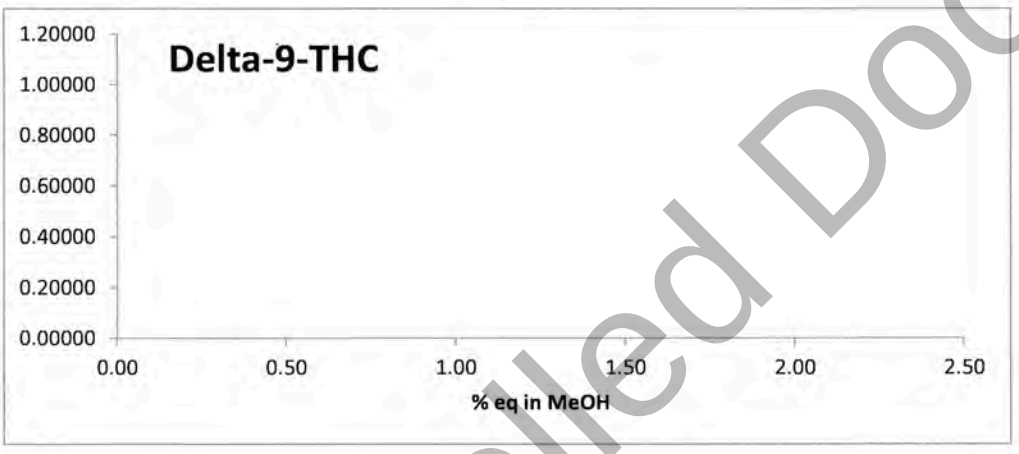
Calibration Date:

delta -9-THC		
% eq.	Area	Rt
2.00		
1.00		
0.50		
0.30		
0.10		
0.05		

Continuing Calibration Verification Date:

delta-9-THC			
% eq.	Area	Rt	% Accuracy
0.30			
0.30			

Acceptable Range: (85-115%)



Sample Results

Sample #	Area	% THC

QC Percent Recoveries

QC	Expected	Actual	% Recovery
Matrix Spike			
LCS			

Matrix Spike and LCS Control Charted Date:_____ Initials: _____

Duplicate %Difference

	%THC	% Difference
Sample		#DIV/0!
Duplicate		

Analyst:_____ Date:_____