THE STATE of ALASKA
GOVERNOR MICHAEL J. DUNLEAVY

Department of Commerce, Community, and Economic Development

ALCOHOL & MARIJUANA CONTROL OFFICE

550 West Seventh Avenue, Suite 1600
Anchorage, AK 99501
Main: 907.269.0350

MEMORANDUM

TO: Chair and Members of the Board
DATE: February 7, 2019

FROM: Erika McConnell
Director, Marijuana Control Board
RE: Peak Analytical, LLC #15590

Peak Analytical, LLC, a marijuana testing facility, is requesting approval of amendment(s) to its operating plan supplemental. Attached is MJ-15 Operating Plan Change application, current MJ-06, and updated SOP Manual.

Alcohol and Marijuana Control Office
550 W 7th Avenue, Suite 1600
Anchorage, AK 99501
marijuana.licensing@alaska.gov
https://www.commerce.alaska.gov/web/amco
Phone: 907.269.0350

Alaska Marijuana Control Board

Form MJ-15: Operating Plan Change

What is this form?

This operating plan change form is required for all marijuana establishment licensees seeking to change a licensed marijuana establishment's existing operating plan, as required by 3 AAC 306.100. With this form, a licensee may request changes to as much or as little as desired of Form MJ-01 and/or the corresponding operating plan supplemental for the establishment's license type. **The required $250 change fee may be made by check, cashier's check, or money order.**

Please complete and submit with this form the pages of Form MJ-01 and/or the corresponding operating plan supplemental that contain sections that you are requesting to change. **All fields must be completed of any page for which you are requesting changes** – upon board approval, the submitted pages will replace those currently on file. If your current, approved operating plan is on the original version of the forms, you may be required to complete and submit the new operating plan forms in their entirety.

The form(s) that I am requesting board approval to change is:

- ☐ **Form MJ-01:** Marijuana Establishment Operating Plan
- ☐ **Form MJ-03:** Retail Marijuana Store Operating Plan Supplemental
- ☐ **Form MJ-04:** Marijuana Cultivation Facility Operating Plan Supplemental
- ☐ **Form MJ-05:** Marijuana Product Manufacturing Facility Operating Plan Supplemental
- ☑ **Form MJ-06:** Marijuana Testing Facility Operating Plan Supplemental

This form must be completed and submitted to AMCO's main office prior to changing existing operations. The licensed establishment's operations may not be altered unless and until the director has given temporary approval or the Marijuana Control Board (MCB) has given final approval of the changes. Please note that licensees seeking to change operating plans for multiple licenses must submit a separate completed copy of this form for each license.

Section 1 – Establishment Information

Enter information for the business seeking to be licensed, as identified on the license application.

Licensee:	Peak Analytical LLC	**MJ License #:**	15590		
License Type:	Marijuana Testing Facility				
Doing Business As:	Peak Analytical LLC				
Premises Address:	2208 Tongass Avenue				
City:	Ketchikan	**State:**	Alaska	**ZIP:**	99901

Received by AMCO 2.5.19

Alaska Marijuana Control Board

Form MJ-15: Operating Plan Change

Section 2 – Summary of Changes

Provide a summary of the changes for which you are requesting approval.

Peak Analytical LLC requests authorization to add terpene analysis to our testing menu. This analysis would be performed utilizing head space gas chromatography with flame ionization detection and a Full Evaporation Technique. Analytes include 19 common terpinoids found in cannabis; see page 34 of the Standard Operating Procedure (SOP) Manual for a detailed list. The list of terpinoid analytes may vary over time as the market changes. The analysis SOP is based on a method published by Restek, the manufacturer of the analytical column used for analysis. Details may be found on pages 31-37 of the Peak Analytical SOP Manual, attached.

The SOP for residual solvent analysis is also based on a method published by Restek and is principally the same as was originally approved by the Board at its June 2017 meeting. Details may be found on pages 23-30 of the Peak Analytical SOP Manual, attached.

The International Organization for Standardization's (ISO) standard number 17025, Section 7.2.1.4 states that "...Methods published either in international, regional or national standards, or by reputable technical organizations, or in relevant scientific texts or journals, or as specified by the manufacturer of the equipment, are recommended. Laboratory-developed or modified methods can also be used."

With the submittal of this form, Peak Analytical LLC is seeking concurrence from the Board that the testing procedures referenced above are scientifically valid per ISO 17025 and Peak Analytical LLC has met the requirement of 3 AAC 306.635 (a)(2).

Section 3 – Declarations

Read each statement below, and then sign your initials in the corresponding box to the right: Initials

The proposed changes conform to all applicable public health, fire, and safety laws. KJ

I understand that any temporary approval granted by the director is pending a final decision by the MCB; therefore, any investment I make, based upon temporary approval, is at my own risk. KJ

As a marijuana establishment licensee, I declare under penalty of unsworn falsification that this form, including all accompanying schedules and statements, is true, correct, and complete.

Signature of licensee

Kara Jurczak
Printed name of licensee

ANGELA K. POOL NOTARY PUBLIC STATE OF ALASKA

Angela K. Pool
Notary Public in and for the State of Alaska.

My commission expires: 05/18/22

Subscribed and sworn to before me this 5TH day of FEBRUARY, 2019.

AMCO Director Review for Temporary Approval Pending Final MCB Decision: Approved ☐ Disapproved ☐

Printed name of Director

Date

Signature of Director

Director Comments:

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Operating Plan Supplemental
Form MJ-06: Marijuana Testing Facility

Section 2 – Prohibitions

Applicants should review 3 AAC 306.610 and be able to answer "Agree" to all items below.

The marijuana testing facility will not:	Agree	Disagree
Sell, deliver, distribute or transfer any marijuana or marijuana product to a consumer, with or without compensation	☑	☐
Allow any person to consume marijuana or marijuana product on its licenses premises	☑	☐

Section 3 – Testing Practices and Procedures

Review the requirements under 3 AAC 306.615, 3 AAC 306.635 – 3AAC 306.645, and 3 AAC 306.660, and identify how the proposed establishment will meet the listed requirements.

Describe each test the marijuana testing facility will offer:

Potency testing will be offered for marijuana, marijuana products and marijuana concentrates. Analytes include THC, THCa, THCv, CBG, CBD, CBDa and CBN. The test specimen will undergo sample preparation prior to analysis using High Performance Liquid Chromatography. Sample preparation generally consists of homogenization, extraction and concentration or dilution. The test will result in a percentage by weight. Total THC will be calculated as:
Total THC=(%THC) + (%THCa)x0.877

Microbial testing will be offered for marijuana, marijuana products and marijuana concentrates. Analytes include shiga-toxin escherichia coli (STEC), salmonella and aspergillus fumigatus, aspergillus flavus, aspergillus niger. Microbial contaminants will be analyzed using standard plate counts. Sample preparation generally consists of homogenization and dilution prior to testing.

Residual solvents testing will be offered for solvent based marijuana concentrates. This analysis will be performed utilizing head space gas chromatography with flame ionization detection. Analytes include butanes, heptanes, benzene, toluene, hexane and total xylenes.

Terpene analysis will be offered for marijuana and marijuana concentrates. This analysis will be performed utilizing head space gas chromatography with flame ionization detection. Analytes include 19 common terpenoids found in cannabis; see page 34 of the Peak Analytical Standard Operating Procedure (SOP) Manual for a detailed list. The list of terpenoid analytes may vary over time as the market changes.

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Operating Plan Supplemental
Form MJ-06: Marijuana Testing Facility

Standard Operating Procedure Manual (3 AAC 306.640):

Applicants for marijuana testing facilities must have a written procedures manual with detailed instructions explaining how to perform each testing method the applicant or marijuana testing facility uses, and minimum standards for each test. Applicants should be able to answer "Agree" to all items below.

The marijuana testing facility will ensure that the standard operating procedure manual:	Agree	Disagree
Is available to each employee at all times	☑	☐
Will cover at least the required procedures listed under 3 AAC 306.640	☑	☐

Describe the marijuana testing facility's standard operating procedure for each test the facility will offer:

Potency Testing:
Plant Matrices and Concentrates:
-Into extraction vial weigh 0.500 g of homogenized sample (Concentrates: weigh 0.100 g of homogenized sample) -Pipette 10 mL of MeOH on top of the sample -Vortex for 4 minutes -Centrifuge 1 minute at 5000 rpm -Filter supernatant with syringe filter into clean transfer vial -Add 990 µl of MeOH to the autosampler vial -Transfer 10 µl of the filtered extract into the autosampler vial, then cap the vial -Mix thoroughly by shaking & analyze via HPLC
Edible Matrices:
-Into extraction vial weigh 0.500 g homogenized sample (Carbonated beverages: add 10 ml degassed sample) -Add 5 ml of reagent water and hydrate for 30-60 mins using orbital rocker -Add 5 ml of a high polar index solvent: Acetonitrile (ACN containing 1% Acetic Acid) or MeOH -Add one QuEChERS Q-sepTM Original Unbuffered Method packet -Vortex for 4 minutes -Centrifuge for 5 minutes at 5000 rpm -Transfer approximately 1 ml of the supernatant to a dSPE tube -Vortex for 1 minute -Centrifuge for 4 minute at 5000 rpm -Add 950 µl of MeOH to the autosampler vial -Transfer 50 µl of the supernatant into the autosampler vial, then cap -Mix thoroughly by shaking & analyze via HPLC

Microbial Testing:
Plant & Edible Matrices (analyzed for STEC)
1.Allow plates (Hardy Diagnostics Compact DryTM EC) to reach room temperature
2.Using one sterile pipet or scoop for each sample, aliquot 1 ml or 1 g of homogenized test sample to a 99 ml pre-filled Dilu-Lok II™ dilution vial to yield a 1:100 dilution. Mix thoroughly by shaking 25 times
3.Transfer approximately 10-15 ml of diluted sample into a sterile centrifuge tube
4.Centrifuge 2 minutes at 5000 rpm or filter with a syringe filter
7.Inoculate sample plates by pipetting 1 ml of each sample dilution directly to the center of a dry plate well
8.Replace plate lids
9.Invert the plates and incubate, upside down with the medium on top, at 35-37 degrees C for 24 hours

See additional space provided. These are abbreviated methods. See attached Standard Operating Procedures Manual for full methods.

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Operating Plan Supplemental
Form MJ-06: Marijuana Testing Facility

Laboratory Testing of Marijuana and Marijuana Products (3 AAC 306.645):

A licensed marijuana testing facility must meet minimum standards for laboratory testing. Applicants should be able to answer "Agree" to all items below.

The marijuana testing facility applicant has:	Agree	Disagree
Read and understands and agrees to the requirements listed under 3 AAC 306.645	☑	☐

Describe the acceptable range of results for each test the marijuana testing facility will offer:

Potency Testing - a sample has passed if it meets the following criteria:
1. less than 5 mg active THC or Delta 9 in a one serving edible
2. less than a cumulative 50 mg active THC or Delta 9 in a multi serving package
3. or any serving in a multiple serving package is less than 20% from the manufacturer's target

Microbial Testing - the test returns a failed result if one colony forming unit of any analyte (shiga-toxin escherichia coli (STEC), salmonella and aspergillus fumigatus, aspergillus flavus, aspergillus niger) is detected in a one-gram sample.

Residual Solvents - must have concentrations less than values stated below for a passing result:
butanes < 800 PPM
heptanes <500 PPM
benzene <1 PPM
toluene <1 PPM
hexane <10 PPM
total xylenes <1 PPM

Terpene Analysis - will be reported in percent by weight for each terpenoid detected. There are no regulatory limits on terpenoid content.

Review of analytical results will ensure tests meet the acceptable range of results for the laboratory. Quality control analyses' will be performed on every sample batch in order to ensure consistency of testing accuracy.

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Operating Plan Supplemental

Form MJ-06: Marijuana Testing Facility

(Additional Space as Needed):

Continuation from Page 3 and the first Page 8

Terpene Testing:
1.Weigh specified amount of homogenized sample into a 20 ml headspace vial
2.Prepare calibration standards and two air blanks (empty vials)
3.Add the samples to the headspace sampler in this order: air blank, standards (high to low for standard curve), sample, air blank
4.Run the analysis including calibration

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Form MJ-06: Marijuana Testing Facility

(Additional Space as Needed):

Continuation from Page 3

Microbial Testing: Concentrates (Salmonella)
1.Allow plates (Hardy Diagnostics Compact DryTM SL) to reach room temperature
2.Label plates with sample identifiers and "blank"
4.Inoculate the control blank with 1 ml sterile water
5.Inoculate sample plates by pipetting 1 g of each sample directly to the dry plate well, keep sample 1 cm from the edge of the well, being careful not to touch the surface of the matrix with the pipet tip.
a.Use one sterile pipet for each sample
b.When the sample is viscous, pipetting the sample on several points on a plate may be needed
6.Pipet 1 ml of sterilized water at the opposite side of the plate from where the specimen was dropped. Once dispensed, the sterile water will automatically diffuse across the surface by capillary action to form a gel; manual spreading of the inoculum is discouraged
8.Invert the plates and incubate, upside down with the medium on top, at 41-43 degrees C for 20-24 hours

Microbial Testing: Concentrates (Aspergillus)
1.Allow plates (Hardy Diagnostics Compact DryTM YMR) to reach room temperature
2.Label plates with sample identifiers and "blank"
4.Inoculate the control blank with 1 ml sterile water
5.Inoculate sample plates by pipetting 1 g of each sample directly to the dry plate well, keep sample 1 cm from the edge of the well, being careful not to touch the surface of the matrix with the pipet tip.
b.When the sample is viscous, pipetting the sample on several points on a plate may be needed
6.Pipet 1 ml of sterilized water at the opposite side of the plate from where the specimen was dropped. Once dispensed, the sterile water will automatically diffuse across the surface by capillary action to form a gel
8.Incubate inverted plates at 25-30 degrees C for 48-72 hours (+/- 2 hours)

Residual Solvents Testing:
1.Weigh 20 mg of oil, wax or other cannabinoid concentrate to a 20 ml headspace vial
2.Prepare calibration standards, at least one air blank (empty vial) and two DMSO blanks
3.Add the samples to the headspace sampler in this order: air blank, DMSO, standards (high to low for standard curve), sample, DMSO
4.Run the analysis including calibration

PEAK ANALYTICAL
STANDARD OPERATING PROCEDURES
MANUAL

REVISION 3

December 2018

Peak Analytical LLC
2208 Tongass Avenue
Ketchikan, Alaska 99901

APPROVAL

SCIENTIFIC DIRECTOR-SIGNATURE: 

SCIENTIFIC DIRECTOR-PRINT: Kara Jurczak

DATE: 12/13/2018

TABLE OF CONTENTS

HOMOGENIZATION

Plant Matrices

Obtain 1-2 g and remove all parts which will not be consumed
Grind to a fine powder with pestle and mortar

Concentrate: Oil, Wax, Honeycomb Wax

No homogenization required

Concentrate: Kief, Dry-Sift, Bubble Hash

Stir or shake until well mixed

Concentrate: Shatter or Brittle/Glassy Items

Grind to a powder with pestle and mortar or break up into tiny pieces, mix well

Hard Candy, Chocolate Bars

Grind with benchtop homogenizer

Baked Goods

Grind with benchtop homogenizer
Dry in oven for 30-60 minutes, stirring occasionally
Grind with pestle and mortar

Gummy, Soft Candy, Caramels

Place in a benchtop homogenizer cup & freeze at -22C for minimum min 30 minutes
Grind with benchtop homogenizer
*Soft candy may require repeating the homogenization procedure

Carbonated Beverages

Degas for 30-minutes on 3-D rocker

Homogenization of samples for Microbial analysis

Aseptically weigh 1 g sample into a sterile vial
Grind with the handheld homogenizer using a clean, sterile tip for each sample
Soft edibles may need to be frozen at -22C for 30-60 minutes before homogenization

POTENCY TESTING

Extraction and Cleanup

- *The remaining extracts should be stored until analysis is completed*
- *If analysis will not occur on the same day the analytical sample should be stored at 4 degrees C*
- *After analysis is completed, re-cap the vial with a new cap to avoid sample evaporation and store at 4 degrees C*

Plant Matrices and Concentrates

1. Mark centrifuge vial with sample identifier
2. Tare vial without cap
3. **Plants**: weigh 0.250 g (0.2750 – 0.2250 g) homogenized sample into vial

   Concentrates: weigh 0.100 g (0.110 – 0.090 g) homogenized sample into vial
   a. *Record exact amount weighed, analyst name, date on Chain of Custody*
4. Add 5 mL of MeOH on top of the sample, then cap
5. Vortex for 1 minute
6. Centrifuge 2 minute at 5000 rpm
7. Filter supernatant with syringe filter into clean transfer vial
8. Write sample identifier on 1.8 ml autosampler vial
9. Dilute at 1:100
   a. Add 990 µl of MeOH to the autosampler vial
   b. Transfer 10 µl of the filtered extract into vial, cap the vial
   c. Mix thoroughly by shaking
10. Use the 1.8 ml autosampler for analysis

Table 1 Example Sequence Table in Chem Station (Flower & Concentrate)

Vial	Sample Name	Method Name	Inj/Vial	Sample Type	Sample Amount (mg)	Multiplier	Dilution
1	Blank	Blank	1	Sample			
2+	Sample ID	Potency-PA	1	Sample	252.3		
92	Wash	Wash-PA	1	Sample			

Edible Matrices

1. Mark centrifuge vial with sample identifier
2. Tare extraction vial without cap
3. **Edibles**: weigh 0.100 g (0.110 – 0.090 g) homogenized sample into vial

   Carbonated beverages: add 1 ml degassed sample into vial

   a. *Record exact amount weighed, analyst name, date on Chain of Custody*
4. Add 1 ml of reagent water and hydrate for minimum 30 mins using orbital rocker
5. Add 1 ml of Acetonitrile Solution (ACN containing 1% Acetic Acid)
6. Add 0.5 g QuEChERS Q-sep™ Original Unbuffered Method salt
7. Vortex for 1 minutes
8. Centrifuge for 10 minutes at 5000 rpm
9. Filter & Dilute according to Table 2:

Table 2 Edibles Dilution Table

Edible Matrix	Filter Supernatant	Dilution	Vol MeOH (µl)	Vol Supernatant (µl)
Gummy & Soft Candy	Into transfer vial	1:10	900	100
Butter	Into transfer vial	1:500	499	1
All Other Edibles	Approx. 1 ml into 1.8 ml vial	1:1	0	1000

10. Mix thoroughly by shaking
11. Use the 1.8 ml autosampler for analysis

Table 3 Example Sequence Table in Chem Station (Edibles)

Vial	Sample Name	Method Name	Inj/Vial	Sample Type	Sample Amount (mg)	Multiplier	Dilution
1	Blank	Blank	1	Sample			
2+	Sample ID	Potency-PA	1	Sample		As Calced	Per SOP
92	Wash	Wash-PA	1	Sample			

$$\text{Multiplier} = \frac{\text{Serving Wt}}{\text{Test Wt}} \div 1000$$

specific to edibles

$$\text{Dilution Factor} = \frac{\text{Total Vol } \textit{(supernatant + dilution solvent)}}{\text{Supernatant Vol}}$$

POTENCY TESTING

Sequence Table Inputs

Flower	
Report:	ESTD%
Test Wt:	mg

Concentrates	
Report:	ESTD%
Test Wt:	mg
***Due to 100 mg test weight the Multiplier is 0.01*	
***Due to the method the Dilution Factor is 100*	
****M x DF cancel each other = no input needed*	

Edibles	
Report:	ESTD
Multiplier = *specific to edibles*	$\frac{\text{Serving Wt}}{\text{Test Wt}} \div 1000$
Dilution Factor =	Per SOP for each type of edible

Report Types				
ESTD%	calculates using:	Test Weight	Multiplier	Dilution Factor
ESTD	calculates using:	-	Multiplier	Dilution Factor

$$\text{Multiplier} = \frac{1}{\text{Test Wt in mg}}$$

$$\text{Dilution Factor} = \frac{\text{Total Vol } \textit{(supernatant + dilution solvent)}}{\text{Supernatant Vol}}$$

POTENCY TESTING

Calibration

Calibration Frequency: Twice per month, after major maintenance, CCV failure, analytical column change or if chromatographic conditions change (i.e. peak identifications change, significant retention time changes).

1. A four-point calibration curve must be diluted according to Table 5 below using Certified Reference Materials (CRM)
    a. *Class-A syringes are used*
    b. *The amount measured must be between 10% and 100% of the syringe volume*
    c. *Syringe rinsing & inoculation is required when switching CRMs*
        i. *Rinse exterior of needle*
        ii. *Rinse syringe with MeOH 3 times*
        iii. *Inoculate syringe with small amount of next CRM*

Table 4 Create 100 µg/ml Working Stock w/ High Conc CRMs

CRM Concentration (µg/ml)	Vol CRM (µl)	Vol MeOH (µl)	Conc of Working Stock (µg/ml)	Vol of Working Stock (µl)
1000	100	900	100	1000
2500	40	960	100	1000

Table 5 Create Calibration Standards using 100 µg/ml CRM or Working Stock

Dilution Factor Info (CRM Vol/Total Vol)	Vol CRM (µl)	Vol MeOH (µl)	Total Vol (ml)	Conc of Cal Std (µg/ml)	Calibration Level
5/100 = .05 = 1:20	20	380	400	**5**	1
10/100 = .1 = 1:10	40	360	400	**10**	2
50/100 = .5 = 1:2	250	250	500	**50**	3
100/100 = 1 = 1:1	400	0	400	**100**	4

Table 6 Create 10ppm CCV using 100 µg/ml CRM or Working Stock

Dilution Factor Info (CRM Vol/Total Vol)	Vol CRM (µl)	Vol MeOH (µl)	Total Vol (ml)	Conc of Cal Std (µg/ml)
10/100 = .1 = 1:10	100	900	1000	10

2. Create your Sequence Table in Chem Station and run the sequence:

Table 7 Create Sequence Table in Chem Station

Vial	Sample Name	Method Name	Inj/Vial	Sample Type	Cal Level	Update RF	Update RT
1	Blank	Blank	1	Sample			
2	Std 5	Potency-PA	1	Calibration	1	Replace	Replace
3	Std 10	Potency-PA	1	Calibration	2	Replace	Replace
4	Std 50	Potency-PA	1	Calibration	3	Replace	Replace
5	Std 100	Potency-PA	1	Calibration	4	Replace	Replace

To *Recalibrate* Once a Calibration Table already exists:

3. Open the calibration data file and select Std 5
    a. Calibration dropdown menu:
        i. Select Recalibrate
            1. Level = 1, select Replace, press OK
4. **Repeat Step 3 for all calibration levels**
5. Adjust peak width windows if necessary:
    a. Calibration dropdown menu:
        i. Calibration Table Options, Select Identification Details (this adds From/To columns to the table
    b. For each compound adjust From/To times to capture entire peak
        i. Add ~.25min to From time to capture the entire peak as the retention time increases over time due to column buildup
6. **Save Method**
7. Chem Station will create a calibration curve for each compound in the calibration table. The calibration curve is typically linear and forced through zero.
8. The correlation coefficient must be > or = 0.995 for each compound. if correlation coefficient is less than 0.995 corrective actions must be taken
    a. Check baseline integrations for improper integration of peaks

b. Wash the column using the wash method and wash sample
c. Backflush column by reversing flow and allowing liquid to flow in waste container
d. Replace guard-column
e. Replace any parts according to manufacturer specifications
f. Re-run calibration curve

To Create a *new* Calibration Table:

9. Open the calibration data file and select Std 100
    a. Calibration dropdown menu:
        i. Select New Calibration Table
            1. Select Automatic Setup, Level = 4, Amount = 100, press OK
                a. Name each compound based on elution order, retention times and CRM manufacturer's chromatogram
10. **Repeat Step 9 for all calibration levels**
11. Perform steps 5 through 8 above
12. **Save Method**
13. Record calibration in log book

Calibration Settings Dropdown Menu *(These are saved in method Potency-PA)*

		Mins	%
Type: Linear			
Origin: Include	Reference Peaks	0.00	5.00
Weight: Equal	Other Peaks	0.00	5.00
Calc Uncalibrated Peaks: No	Units: ug/ml		
# Peaks Missing: Partial Calibration	Signal: MWD Sig 230, 16 REF, 360, 100		

POTENCY TESTING

Quality Control Checks

1. Check for steady baseline conditions before running the batch, record on baseline log sheet
2. The sample batch must include a Continuing Calibration Verification standard (CCV) after every 20 samples. *The initial calibration is considered as a valid CCV*
    a. The concentration of the CCV is a mid-point of the calibration curve; either 10 µg/ml or 50 µg/ml
    b. CCV criteria: The CCV calculated amount for each cannabinoid must not deviate more than 10% from the known concentration

    $$\text{Percent Deviation} = 100 * \frac{(\mu g/ml \text{ CCV Readout} - \mu g/ml \text{ known concentration})}{(\mu g/ml \text{ known concentration})}$$

    c. If the CCV fails, corrective action must be taken:
        i. All samples prior to and after the failed CCV must be re-analyzed with a valid calibration
        ii. Follow the corrective actions as stated in the Quality Manual
3. Load chromatogram in the Data Analysis tab
    a. Review the blank sample for irregularities
    b. Check baseline integrations for irregular integration of peaks
    c. Review analysis report and ensure amounts are within the calibrated range of 5.0 to 100.0 µg/ml
        i. If reporting in %, you must compare peak areas of sample results to peak areas in the calibration table

Eluent Order
CBV (Cannabivarin)
CBD-A (Cannabidiolic Acid) *R*
CBD (Cannabidiol) *R*
CBG (Cannabigerol)
CBN (Cannabinol) *R*
THC (delta 9 Tetrahydrocannabinol) *R*
THC-A (Tetrahydrocannabinolic Acid) *R*

POTENCY TESTING

Interpretation

1. For flower and concentrates ChemStation will read out the % of each cannabinoid
2. For edibles ChemStation will read out the mg of each cannabinoid per serving of edible
3. THC_{Total} = THC + 0.8770*(THC-A)
4. CBD_{Total} = CBD + 0.8770*(CBD-A)
5. If the reported value is zero, record as ND
6. If the reported value is <1 ppm (0.0001%), record as <LOD

General calculation for the percent of each cannabinoid if the read-out is given in µg/ml:

$$\text{Percent \%} = 100 * \text{Dilution Factor} * \frac{\text{ml final volume}}{\text{mg extracted}} * \frac{1\ \text{mg}}{1000\ \mu\text{g}} * \frac{\text{read-out}\ \mu\text{g}}{\text{ml}}$$

Example: For a 500 mg sample that has been extracted in 10 ml MeOH and analyzed at 1:100 dilution with a read-out (found) concentration of 75.0 µg/ml

$$\textit{Percent \%} = 100 * 100 * \frac{10\ \text{ml}}{500\ \text{mg}} * \frac{1\ \text{mg}}{1000\ \mu\text{g}} * \frac{75.0\ \mu\text{g}}{\text{ml}} = 15.00\%$$

Example: If the same sample is analyzed with a dilution factor of 100 entered into chemstation

$$\textit{Percent \%} = 100 * \frac{10\ \text{ml}}{500\ \text{mg}} * \frac{1\ \text{mg}}{1000\ \mu\text{g}} * \frac{7500.0\ \mu\text{g}}{\text{ml}} = 15.00\%$$

CONVERSIONS

PPM = mg/l = µg/ml
PPM = mg/kg = µg/g
1% = 10,000 ppm

POTENCY TESTING

Instrumentation Setup

Chromatographic Conditions for Potency Analysis

Apparatus: Agilent Technologies Model 1100 HPLC with binary pump, degasser, auto-sampler and thermostatic column compartment.
Column: Restek Raptor ARC-18
Column Temperature: 50 Degrees C
Injection Volume: 5 or 10 µL (more volume might be need for certain analyses)
Wavelength: 230 nm

Mobile Phase:
A – H_20 (0.1% Formic Acid)
B – Acetonitrile (0.1% Formic Acid)
Flow Rate: 1.5 ml/min

Time:	%A	%B
0.00	25	75
7.00	0	100
11.00	0	100
11.10	25	75

Note:
There will be a hold time at 100% B to ensure that the column is clean for the next injection. The pumps ramp up/down between time periods.

MOBILE PHASE PREPARATION

- *Measurements shall be made using the bottom of the meniscus*
- *Allow all mobile phases to equilibrate to room temperature prior to use in HPLC*
- *Once new mobile phase is connected to HPLC, run pump to flush old mobile phase through the system and allow baseline conditions to stabilize prior to analysis*
- *Record mobile phase type and date made in log book*

Line B1 – Acetonitrile w/ 1% Formic Acid

1. Measure exactly 1 liter ACN using a 1 liter volumetric flask
2. Transfer to a clean 2 liter mobile phase bottle
3. Add 2 ml Formic Acid to the mobile phase bottle using a Hamilton syringe or equivalent
4. Measure exactly 1 liter ACN using a 1 liter volumetric flask and add it to the mobile phase bottle
5. Cap mobile phase bottle and mix thoroughly

Line A1 – HPLC Water w/ 1% Formic Acid

1. Add approximately 0.5 liter HPLC Water to a 1 liter volumetric flask
2. Add 1 ml Formic Acid using a Hamilton syringe or equivalent
3. Add HPLC Water to flask until reaching a final volume of 1 liters
4. Cap and mix thoroughly
5. Transfer to a clean 1 liter mobile phase bottle

Line B2 – Acetonitrile Neat

1. Add approximately 1 liter ACN to a clean 1 liter mobile phase bottle

Line A2 – HPLC Water Neat

1. Add approximately 1 liter HPLC Water to a clean 1 liter mobile phase bottle

HPLC BEST PRACTICES

- Always use HPLC grade solvents for mobile phase, cleaning, and troubleshooting.
- Always use clean glass bottles for all solvents used on the instrument.
  Replace all solvents at least every two weeks, depending on solvent type replacement of solvents might need to be more frequent.
- Always record maintenance activities in log book.

Daily Procedures

- Ensure that there is a steady baseline and column is conditioned prior to starting Analysis.
    - Pump pressure should be consistent prior to analysis and can be achieved by running for at least 30 minutes prior to analysis.
    - Allow the detector to warm up for at least 30 minutes prior to analysis.

Weekly Maintenance

- Purge the pump after sitting for a week and after changing mobile phase.
    - Purge is considered sufficient when no bubbles are coming out of the waste line.
- Once per week flush all pump channels with 100% water to remove build up, do NOT pump through the column, pump to waste (Only necessary if your mobile phase contains salt or acid).
- Run a wash sample with the wash method once per week (min) or if pressure increases.

Bi-weekly or Longer Maintenance

- Needle wash-change MeOH every two weeks.
- Turn off computer every two weeks to purge temporary files created by ChemStation.
- Blank-change MeOH every couple of months.
- Gold seal-replace every year or as needed.
- Filter under gold seal-replace every year or as needed; this is critical and can cause pressure issues; filter should be white and clean.
- Outlet check balls-these have gold seals; replace every year or as needed.
- Pump seals-replace every year or as needed.
- Active inlet cartridges-replace as needed; indicated by unstable pump pressure or inability to reach proper pressure.
- UV Lamp will last thousands of hours; leave on M-F; LC self-test can indicate health of lamp.

System Storage

- If powering down the entire system for an extended period, flush the entire system with water (without a column) to remove any buffer build-up. *Only necessary when using salty buffers such as phosphate or borate.
- When removing columns for storage flush the column in the suggested storage solvent (found in column manual), typically this is either 50/50 methanol/water OR 50/50 isopropanol/water.
    - Make sure to seal column ends after removing.

New Column Conditioning

- Start flow slow to flush out, then gradually bring up to normal operating conditions

MICROBIAL TESTING

All tools shall be sterile and all processes shall be performed aseptically
All tools and pipettes shall be used for one unique sample only

Sample Preparation

1. Add 9 ml Dilu-Lok II™ sterile water to a vial containing 1 g of homogenous sample, shake thoroughly to get a homogenous mixture. *May use Butterfield's phosphate-buffered water in lieu of Dilu-Lok II™*

Analysis (Culture Plating)

Shiga Toxin E. Coli (STEC)

Indicator plates: Hardy Diagnostics Compact Dry™ EC

2. Allow plates to reach room temperature
3. Label plates with sample identifiers and dilution factors, label one plate as "blank"
4. Remove plate lids
5. Inoculate the control blank with 1 ml sterile water (one blank per batch of microbial tests)
6. Inoculate sample plates by pipetting 1 ml of each sample mixture directly to the center of a dry plate well, being careful not to touch the surface of the matrix with the pipet tip. *Once dispensed, the sample will automatically diffuse across the surface by capillary action to form a gel; manual spreading of the inoculum is discouraged*
7. Replace plate lids
8. Invert the plates and incubate, upside down with the medium on top, at 35-37 degrees C for 24 hours
9. After incubation read the plates
    a. *E. coli* O157 (STEC) develops pink-purple colonies on Compact Dry™ EC, similar to other members of the coliform group
    b. A passing test has less than 1 CFU/g of pink-purple colonies
        i. Ignore blue colonies
    c. If the plate has 1 or more pink-purple colonies proceed to step 10

Selective Isolation Plates: Hardy Diagnostics MacConkey Agar with Sorbitol

Hardy Diagnostics MacConkey Agar with Sorbitol is to be used as a selective and differential medium for the detection of enterohemorrhagic Escherichia coli O157:H7

1. Allow plates to reach room temperature
2. Label plates with sample identifiers, label one plate as "blank"
3. Remove plate lids
    a. *The agar surface should be dry before inoculating*
4. From the isolated colonies on the indicator plates (presumptive pure cultures), use an inoculation loop to collect and transfer a single colony for further isolation to the MacConkey plate
    a. *NOTE: ONLY PINK-PURPLE COLONIES NEED TO BE ASSESSED*
5. Spread the isolated colony into quadrants for further isolation.
    a. *If isolated colonies do not appear (eg TNTC) re-isolate colonies from sample using a higher dilution factor*
6. Incubate plates aerobically for 24 hours at 37 degrees C
7. After incubation read the plates
    a. *E. coli* O157 (STEC) develops colorless colonies on MacConkey Agar with Sorbitol, similar to other E. Coli species
    b. A passing test has less than 1 CFU/g of colorless colonies
        ii. Ignore pink colonies
    c. If the plate has 1 or more colorless colonies proceed to step 18

2nd Confirmation Kit: Hardy Diagnostics E. ColiPRO O157 Kit

The Hardy Diagnostics E. coliPRO™ O157 Kit provides a rapid latex agglutination method for the detection of E. coli serogroup O157 antigen from colonies isolated from laboratory culture media. In general, the Latex Test should be performed on isolated non-sorbitol-fermenting colonies that are 18 to 24 hours old. Isolates can be taken from MacConkey Agar with Sorbitol or CT-SMAC Agar plates

1. Allow all reagents to come to room temperature for at least 10 minutes prior to use
2. Place one drop of sterile Saline within the circle on the test card

3. Select 1-4 well-isolated colonies from the MacConkey agar surface
4. Create an emulsion of the colonies by mixing the Saline on the test card
5. Mix the Latex Reagents, by inverting the tubes, prior to use. Dispense 1 drop of E. coliPRO™ O157 Latex Reagent onto a test circle on the test card.
    a. *Important: Do not allow the Latex Reagent bottle to come into contact with the organism suspension*
6. Mix the Latex Reagent and the organism suspension with the wooden sticks provided, using the complete area of the circle
    a. A new stick should be used for each reagent
7. Gently hand-rock the entire card, allowing the mixture to flow slowly over the ring area
8. For up to 2 minutes, under normal lighting conditions, observe for agglutination (strong clumping) of the latex particles
    a. All organisms yielding a rapid and strong clumping of the latex particles in under 2 minutes should be retested with the Negative Control Latex Reagent
        iii. Repeat steps 4-8 with the Negative Control Latex Reagent

<u>Negative Results:</u>

- If no visible agglutination of the latex particles is observed in any of the reaction circles, there is no need to continue testing with the Negative Control Latex Reagent
    - This yields a passing test result

<u>Positive Results:</u>

- If the organism agglutinates with the Latex Test Reagent and fails to agglutinate with the Negative Control Latex Reagent, this indicates the identification of E. coli serotype O157
    - This yields a failing test result
- If the organism agglutinates with the Latex Test Reagent and also with the Negative Control Latex Reagent, this is a false-positive reaction due to autoagglutination or cross reaction of the strain

- This yields a passing test result

Salmonella

Enrichment

1. Label sterile vials with sample identifiers
2. Add 1 ml of sample mixture to vial
3. Add 9 ml of Buffered Peptone Water to the vial
4. Incubate 20-24 hours at 35-37 degrees C

Identification plates: Hardy Diagnostics Compact Dry™ SL

1. Allow plates to reach room temperature
2. Label plates with sample identifiers
3. Remove plate lids
4. Inoculate sample plates by pipetting 0.1 ml of enriched sample directly to the dry plate about 1 cm from the edge of the tray, do not touch the plate with pipet tip. *The dispensed sample will remain at the point of inoculation and will not spread across the whole plate*
5. Pipet 1 ml of sterile water at the opposite side of the plate from where the specimen was dropped. *Once dispensed, the sterile water will automatically diffuse across the surface by capillary action to form a gel; manual spreading of the inoculum is discouraged*
6. Replace plate lids
7. Incubate, upside down with the medium on top, for 20-24 hours at 41-43 degrees C
8. After incubation read the plates against a white background
9. Only count Salmonella colonies
    a. Green or blue-green colonies with or without black centers and on a yellow background are indicative of Salmonella

b. Bacteria other than Salmonella may grow and produce a yellow background at the site of inoculation, such as Pseudomonas spp., but will not produce green colonies

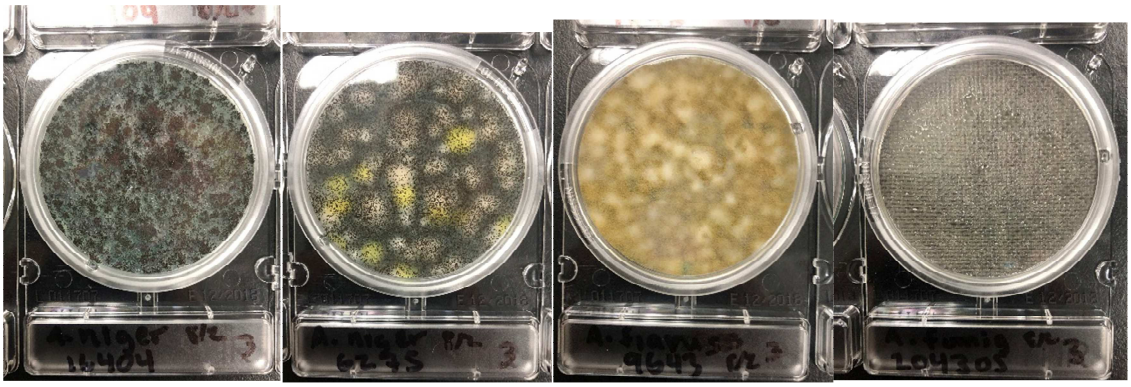
10. A passing test has less than 1 CFU/g of Salmonella colonies

Aspergillus Niger, Flavus, Fumigatus (Mold)

Indicator plates: Hardy Diagnostics Compact Dry™ YMR

Selective Hardy Plates are used for detecting yeasts and molds.

1. Allow plates to reach room temperature
2. Label plates with sample identifiers
3. Remove plate lids
4. Inoculate sample plates by pipetting 1 ml of sample mixture directly to the center of the plate. Do not touch plate with pipet tip. *Once dispensed, the sample will diffuse across the plate to form a gel*
5. Replace plate lids
6. Incubate inverted plates for 48-72 hours (+/- 2 hours) at 25-30 degrees C
7. After incubation read the plates against a white background
8. If the entire growth area is green/blue dilute the sample
9. Blue colored colonies indicate yeast
    a. Ignore blue colonies
10. Cottony colonies with characteristic colors are molds
    a. A passing test has less than 1 CFU/g of mold characteristic colonies
    b. If there are 1 or more colonies characteristic of mold proceed to step 9



Asper. Niger *Asper. Niger* *Asper. Flavus* *Asper. Fumigatus*

Selective Isolation Plates: Hardy Diagnostics Dichloran Rose Bengal Chloramphenicol Agar "Rose Bengal"

Rose Bengal Chloramphenicol Agar is recommended for the selective isolation and enumeration of yeasts and molds from environmental materials and foodstuffs. Chloramphenicol inhibits the growth of bacteria additionally to Rose Bengal, which restricts the height and size of mold colonies so that slow-growing fungi are not overgrown by more rapidly growing species.

1. Remove as many plates from the sleeve as you have presumptive colonies, allow plates to reach room temperature
2. Label plates with sample identifiers, label one plate as "blank"
3. Remove plate lids
4. From the isolated colonies (presumptive pure cultures), using an inoculation loop collect and transfer a single colony for further isolation to a Rose Bengal media plate. *NOTE: ALL TYPES OF COLONIES NEED TO BE ASSESSED*
5. Spread the isolated colony into quadrants for further isolation. *If isolated colonies do not appear (eg TNTC) re-isolate colonies from sample*
6. Incubate at 22 to 25 degrees C and examine plates after 3, 4 and 5 days or until the fruiting body appears for identification
7. Examine the colonies Macroscopically and Microscopically to determine species. Use the Microscopy references

8. Upon Macroscopic and Microscopic examination, a passing test has less than 1 CFU/g of Aspergillus Niger, Flavus or Fumigatus

Disposal of Microbial Testing Media

1. Store used media in the biohazard bin lined with an autoclave bag
2. Autoclave the bag of used media at 121 degrees C for 20 minutes
3. Dispose of sterilized bag in trash

RESIDUAL SOLVENTS TESTING

Calibration

Calibration Frequency: Calibrate before each sample batch

1. A seven-point calibration curve must be created according to Table 8 & 8a below using Certified Reference Materials (CRM)
2. Use only gas tight Hamilton syringe or equivalent
3. The amount measured must be between 10% and 100% of the syringe volume
4. Syringe inoculation with CRM is required
5. The standard should be prepared in a solvent less volatile than the analytes or the solvent used in the CRM
    a. Acceptable solvents are dimethyl sulfoxide (DMSO) and dimethyl acetamide (DMA)
        i. DMSO shall be tested for interference before used in standards
6. Cap vial immediately after dispensing CRM or prepare through septa
7. After preparation of calibration standards, rinse syringe 3 times in MeOH then bake in oven for 15 minutes

Table 7 Create 100 µg/ml Working Stock w/ High Conc CRMs

CRM Concentration (µg/ml)	Vol CRM (µl)	Vol DMSO (µl)	Conc. of Working Stock (µg/ml)
1000	30	270	100

Table 8 Create Low Conc. Calibration Standards using 100 µg/ml CRM or Working Stock

Dilution Factor Info (CRM Vol/Total Vol)	Vol CRM (µl)	Conc of Cal Std (µg/ml)	Calibration Level
1/100 = .01 = 1:100	1	**1**	1
5/100 = .05 = 1:20	5	**5**	2
25/100 = .25 = 1:4	25	**25**	3
50/100 = .5 = 1:2	50	**50**	4
100/100 = 1 = 1:1	100	**100**	5

Table 8(a) Create High Conc. Calibration Standards using 1000 µg/ml CRM

50/1000 = .05 = 1:20	50	**500**	6
100/1000 = .1 = 1:10	100	**1000**	7

8. Create a Sequence Table in ChemStation and run the sequence:

Table 9 Sequence Table in ChemStation

Vial	Sample Name	Method Name	Inj/ Vial	Sample Type	Cal Level	Update RF	Update RT
1	Air Blank	Blank-PA	1	Sample			
2	DMSO Blank	ResSolv-PA	1	Sample			
3	Std 1	ResSolv-PA	1	Calibration	1	Replace	Replace
4	Std 5	ResSolv-PA	1	Calibration	2	Replace	Replace
5	Std 25	ResSolv-PA	1	Calibration	3	Replace	Replace
6	Std 50	ResSolv-PA	1	Calibration	4	Replace	Replace
7	Std 100	ResSolv-PA	1	Calibration	5	Replace	Replace
8	Std 500	ResSolv-PA	1	Calibration	6	Replace	Replace
9	Std 1000	ResSolv-PA	1	Calibration	7	Replace	Replace
10+	Customer Sample	ResSolv-PA	1	Sample			
#	Bake Blank	Bake-PA	1	Sample			

9. Adjust Headspace Autosampler stop vial to # (above) and run Method 1

To *Recalibrate* Once a Calibration Table already exists:

10. Open the calibration data file and select Std 1
    a. Calibration dropdown menu:
        i. Select Recalibrate
            2. Level = 1, select Replace, press OK
11. **Repeat Step 10 for all calibration levels**
12. Adjust peak width windows if necessary:
    a. Calibration dropdown menu:
        i. Calibration Table Options, Select Identification Details (this adds From/To columns to the table
    b. For each compound adjust From/To times to capture entire peak
        i. Add ~.25min to From time to capture the entire peak as the retention time increases over time due to column buildup
13. **Save Method**
14. Chem Station will create a calibration curve for each compound in the calibration table. The calibration curve is typically linear and forced through zero.
15. The correlation coefficient must be > or = 0.995 for each compound. if correlation coefficient is less than 0.995 corrective actions must be taken
    a. Check baseline integrations for improper integration of peaks
    b. Bake off the GC and HS
    c. Replace any parts according to manufacturer specifications
    d. Re-run calibration curve

To Create a *new* Calibration Table:

16. Open the calibration data file and select Std 1000
    a. Calibration dropdown menu:
        i. Select New Calibration Table
            1. Select Automatic Setup, Level = 7, Amount = 1000, press OK

a. Name each compound based on elution order, retention times and CRM manufacturer's chromatogram

17. **Repeat Step 16 for all calibration levels**
18. Perform steps 12 through 15 above
19. **Save Method**
20. Record calibration in log book

RESIDUAL SOLVENTS TESTING

Analysis

1. Mark 20 ml headspace vial with sample identifier
2. Tare vial without cap
3. Accurately weigh 0.02 g of oil, wax or other concentrate into vial
    a. *Record exact amount weighed, analyst name, date on Chain of Custody*
4. Prepare one air blank (empty vial) and one DMSO (or other solvent used) blank (10 µl)
    b. *Prior air blanks may be reused as long as septa is sound*
5. Add the samples to the headspace sampler in this order: air blank, DMSO, calibrations standards (low to high), samples, bake blank
6. Run the analysis
7. Calibrate the system per Residual Solvents Testing – Calibration Section
8. If system suitability is met, record the residual solvent concentrations for the peaks identified
9. Identify and integrate the peaks
    a. The elution order is
        i. Isobutane
        ii. Butane
        iii. N-hexane
        iv. Benzene
        v. Heptane
        vi. Toluene
        vii. Xylene
        viii. DMSO or DMA diluent peak
            2. Except the diluent peak, all peaks should elute within 3 to 7 minutes

RESIDUAL SOLVENTS TESTING

Quality Control Checks

1. Check for steady baseline conditions before running the batch, record on baseline log sheet
2. Ensure calibration meets stated requirement listed in the Calibration Section of this manual
    a. If calibration fails, follow the corrective actions as stated in the Quality Manual
3. Load chromatogram in the Data Analysis tab
    a. Review the blank sample for irregularities
    b. Check baseline integrations for irregular integration of peaks
    c. Review analysis report and ensure peak areas are within the calibrated range of 1.0 to 1000.0 µg/ml found in the calibration table

RESIDUAL SOLVENTS TESTING

Interpretation

If a readout concentration is not available, concentrations may be calculated using the following:

$y = m * x + b$

y = peak area of solvent / peak area of STD
m = slope of the line
x = concentration of solvent / concentration of STD
b = intercept

Solving the equation for the unknown concentration of solvent yields:

$$\text{Concentration of Solvent} = \frac{[y-b] * [\text{STD concentration}]}{m}$$

The linear plot determines the values of m and b, the value of STD concentration is known and included in the method, and the value of y is determined from the sample runs.

For each sample run the instrument will calculate and report the concentration of residual solvent in µg/ml. This value will be used to determine the concentration of residual (µg/ml) in the original gas sample using the following equation:

$$\text{Concentration of solvent} = \frac{[\text{conc gas in extract sample}] * [\text{volume STD}]}{\text{mass of original sample used}}$$

RESIDUAL SOLVENTS TESTING

Regulatory Limits [3 AAC 306.645(B)(3)]

Substance	**Acceptable Limit per Gram**
Butanes	Less than 800 PPM (µg/g)
Heptanes	Less than 500 PPM (µg/g)
Benzene	Less than 1 PPM (µg/g)
Toluene	Less than 1 PPM (µg/g)
Hexane	Less than 10 PPM (µg/g)
Total xylenes	Less than 1 PPM (µg/g)

CONVERSIONS

PPM = mg/l = µg/ml
PPM = mg/kg = µg/g
1% = 10,000 ppm

RESIDUAL SOLVENTS TESTING

Instrumentation Setup (HS GC-FID Full Evaporation Technique [FET])

GC-FID Settings (Agilent 6890)

Column: RXi-624Sil MS, 30 m, 0.32 mm ID, 1.8 µM Cat# 13870
Sample: Residual Solvent mix
Diluent: Dimethyl sulfoxide (DMSO)
Injector Port: 250 °C with 10:1 split
Liner: 2.0 mm ID straight inlet liner
Carrier Gas: Nitrogen using constant flow mode
Linear Velocity: 64 cm/sec
Carrier Flow Rate: 55.3 ml/min
Oven: 35 °C for 1.5 min, ramp to 300 °C at 30 °C /min and hold 2 min.
Detector: FID at 350 °C
Make-up Gas: Nitrogen
Make-up Gas Flow Rate: 45 ml/min
Hydrogen Flow: 40 ml/min
Air Flow: 450 ml/min
Data Rate: 20 Hz

Headspace Sampler Settings (Tekmar 7000) – Method 1

Sample Temp: 140°C
~~Needle Temp: 140°C~~ N/A on this HS
Valve Oven: 160 °C
Loop: 160 °C
Transfer Line: 160 °C
Vial Pressure: 20 psi
GC Cycle Time: 25 min
(time for GC to cool down after a run)
Vial Eq. Time: 30 min
Pressurize Time: 5.0 min
Equilibration Pressure: 0.05 min
Loop Fill Time: 2.0 min
Loop Eq. Time: 0.1 min
Inj. Time: 1.0 min
*(these settings may be modified to adjust instrument response)

TERPENE PROFILING

Calibration

Calibration Frequency: Once per month, after major maintenance (change septa or inlet liner), analytical column change, CCV failure or if chromatographic conditions change (i.e. peak identifications change, significant retention time changes, losing sensitivity).

1. A five-point calibration curve must be created according to Table 11 below using Certified Reference Materials (CRM)
    - *b. Use only gas tight Hamilton syringe or equivalent*
    - *c. The amount measured must be between 10% and 100% of the syringe volume*
    - *d. Syringe inoculation with CRM is required*
    - *e. The standard should be prepared in the same solvent as the CRM*
        - *i. If DMSO, it shall be tested for interference before use*
    - *f. Cap vial immediately after dispensing CRM or prepare through septa*
    - *g. After preparation of calibration standards, rinse syringe 3 times in MeOH then bake in oven for 15 minutes*

Table 10 Create 100 µg/ml Working Stock w/ High Conc CRMs

CRM Concentration (µg/ml)	Vol CRM (µl)	Vol Solvent (µl)	Conc. of Working Stock (µg/ml)
1000	30	270	100
2500	12	288	100

Table 11 Create Calibration Standards using 100 µg/ml CRM or Working Stock

Dilution Factor Info (CRM Vol/Total Vol)	Vol CRM (µl)	Conc of Cal Std (µg/ml)	Calibration Level
1/100 = .01 = 1:100	1	**1**	1
5/100 = .05 = 1:20	5	**5**	2
25/100 = .25 = 1:4	25	**25**	3
50/100 = .5 = 1:2	50	**50**	4
100/100 = 1 = 1:1	100	**100**	5

2. Create a Sequence Table in ChemStation and run the sequence:

Table 12 Sequence Table in ChemStation

Vial	Sample Name	Method Name	Inj/ Vial	Sample Type	Cal Level	Update RF	Update RT
1	Air Blank	Blank-PA	1	Sample			
2	Std 1	Terp-PA	1	Calibration	1	Replace	Replace
3	Std 5	Terp-PA	1	Calibration	2	Replace	Replace
4	Std 25	Terp-PA	1	Calibration	3	Replace	Replace
5	Std 50	Terp-PA	1	Calibration	4	Replace	Replace
6	Std 100	Terp-PA	1	Calibration	5	Replace	Replace
7+	Customer Sample	Terp-PA	1	Sample			
#	Bake Blank	Bake-PA	1	Sample			

3. Adjust Headspace Autosampler stop vial to # (above) and run Method 1

To *Recalibrate* Once a Calibration Table already exists:

4. Open the calibration data file and select Std 1
    a. Calibration dropdown menu:
        i. Select Recalibrate
            1. Level = 1, select Replace, press OK
5. **Repeat Step 4 for all calibration levels**
6. Adjust peak width windows if necessary:
    a. Calibration dropdown menu:
        i. Calibration Table Options, Select Identification Details (this adds From/To columns to the table
    b. For each compound adjust From/To times to capture entire peak
        i. Add ~.25min to From time to capture the entire peak as the retention time increases over time due to column buildup
7. **Save Method**
8. Chem Station will create a calibration curve for each compound in the calibration table. The calibration curve is typically linear and forced through zero.

9. The correlation coefficient must be > or = 0.990 for each compound. if correlation coefficient is less than 0.990 corrective actions must be taken
    a. Check baseline integrations for improper integration of peaks
    b. Bake off GC and HS
    c. Replace any parts according to manufacturer specifications
    d. Re-run calibration curve

To Create a *new* Calibration Table:

10. Open the calibration data file and select Std 100
    a. Calibration dropdown menu:
        i. Select New Calibration Table
            1. Select Automatic Setup, Level = 5, Amount = 100, press OK
                a. Name each compound based on elution order, retention times and CRM manufacturer's chromatogram
11. **Repeat Step 10 for all calibration levels**
12. Perform steps 6 through 9 above
13. **Save Method**
14. Record calibration in log book

TERPENE PROFILING

Analysis

Plant Matrices and Concentrates

1. Mark 20 ml headspace vial with sample identifier
2. Tare vial without cap
3. **Plants**: weigh 0.1 g (0.110 – 0.090 g) homogenized sample into vial
   Concentrates: weigh 0.01 g (0.011 – 0.009 g) homogenized sample into vial
   a. *Record exact amount weighed, analyst name, date on Chain of Custody*
4. Prepare one air blank (empty vial) and bake blank (empty vial)
   a. *Prior blanks may be reused if septa is sound*
5. Add the samples to the headspace sampler in this order: air blank, calibrations standards (low to high), samples, bake blank
6. Run the analysis
7. Calibrate the system per Terpene Profiling – Calibration Section
8. If system suitability is met, record the terpene concentrations for the peaks identified
   a. Identify and integrate the peaks
      i. The elution order is
      ii. Alpha-Pinene
      iii. Camphene
      iv. (-)-beta-Pinene
      v. beta-Myrcene
      vi. delta-3-carene
      vii. alpha-Terpinene
      viii. p-Cymene
      ix. d-Limonene
      x. Ocimene
      xi. gamma-Terpinene
      xii. Terpinolene
      xiii. Linalool
      xiv. (-)-Isopulegol
      xv. Geraniol
      xvi. beta-Caryophyllene
      xvii. alpha-Humulene
      xviii. Nerolidol
      xix. (-)-Gauiol
      xx. (-)-alpha-Bisabolol
      xxi. Isopropanol or other diluent peak
      xxii. Except the diluent peak, all peaks should elute within 6 to 16 minutes

TERPENE PROFILING

Quality Control Checks

1. Check for steady baseline conditions before running the batch, record on baseline log sheet
2. The sample batch must include a Continuing Calibration Verification standard (CCV) after every 20 samples. *The initial calibration is considered as a valid CCV*
    a. The concentration of the CCV is a mid-point of the calibration curve; either 25 µg/ml or 50 µg/ml
    b. CCV criteria: The CCV calculated amount for each cannabinoid must not deviate more than 10% from the known concentration

    $$\text{Percent Deviation} = 100 * \frac{(\mu g/ml \text{ CCV Readout} - \mu g/ml \text{ known concentration})}{(\mu g/ml \text{ known concentration})}$$

    c. If the CCV fails, corrective action must be taken:
        i. All samples prior to and after the failed CCV must be re-analyzed with a valid calibration
        ii. Follow the corrective actions as stated in the Quality Manual
3. Load chromatogram in the Data Analysis tab
    a. Review the blank sample for irregularities
    b. Check baseline integrations for irregular integration of peaks
    c. Review analysis report and ensure amounts are within the calibrated range of 1.0 to 100.0 µg/ml
        i. If reporting in %, you must compare peak areas of sample results to peak areas in the calibration table

TERPENE PROFILING

Interpretation

If a readout concentration is not available, concentrations may be calculated using the following:

$y = m * x + b$

y = peak area of terp / peak area of STD
m = slope of the line
x = concentration of terp / concentration of STD
b = intercept

Solving the equation for the unknown concentration of terpene yields:

$$\text{Concentration of Solvent} = \frac{[y-b] * [\text{STD concentration}]}{m}$$

The linear plot determines the values of m and b, the value of STD concentration is known and included in the method, and the value of y is determined from the sample runs.

For each sample run the instrument will calculate and report the concentration of terpenes in µg/ml. This value will be used to determine the concentration of terpene (µg/ml) in the original gas sample using the following equation:

$$\text{Concentration of terpene} = \frac{[\text{conc gas in extract sample}] * [\text{volume STD}]}{\text{mass of original sample used}}$$

CONVERSIONS

PPM = mg/l = µg/ml
PPM = mg/kg = µg/g
1% = 10,000 ppm

TERPENE PROFILING

Instrumentation Setup (HS GC-FID Full Evaporation Technique [FET])

GC-FID Settings (Agilent 6890)

Column: RXi-624Sil MS, 30 m, 0.32 mm ID, 1.8 µM Cat# 13870
Sample: Terpenes Mix / Client Samples
Diluent: Varies-matches CRM solvent
Injector Port: 250 °C with 10:1 split
Liner: 2.0 mm ID straight inlet liner
Carrier Gas: Nitrogen using constant flow mode
Linear Velocity: 61 cm/sec
Carrier Flow Rate: 48.8 ml/min
Oven: 60 °C for 1.5 min, ramp to 300 °C at 12.5 °C /min and hold 2 min.
Detector: FID at 350 °C
Make-up Gas: Nitrogen
Make-up Gas Flow Rate: 45 ml/min
Hydrogen Flow: 40 ml/min
Air Flow: 450 ml/min
Data Rate: 20 Hz

Headspace Sampler Settings (Tekmar 7000) – Method 1

Sample Temp: 140°C
~~Needle Temp: 140°C~~ N/A on this HS
Valve Oven: 160 °C
Loop: 160 °C
Transfer Line: 160 °C
Vial Pressure: 20 psi
GC Cycle Time: 25 min
(time for GC to cool down after a run)
Vial Eq. Time: 30 min
Pressurize Time: 5.0 min
Equilibration Pressure: 0.05 min
Loop Fill Time: 2.0 min
Loop Eq. Time: 0.1 min
Inj. Time: 1.0 min
*(these settings may be modified to adjust instrument response)

GC-FID & HEADSPACE BEST PRACTICES

- Always record maintenance activities in log book
- Keep the GC powered on whenever possible; at the end of a sequence, set oven temperature to 100°C and maintain constant flows through the inlet, column and detector

Shutdown for 1 week or more

- Bake off GC using Bake-PA method and a blank vial
- Bake off HS using HS Method 3 and a blank vial
  - Make sure to bake off GC and HS at the same time
- Turn oven temp down to 10 C and let it cool below 35 C before turning off
- Once baked-out and oven cooled, power down ChemStation, headspace, GC and turn off all gas cylinders

Daily Analysis Procedures

- Verify gas flows, pressures, and temperatures of all GC and HS components prior to starting a sequence
- Always bake off at the end of a run using Bake-PA method and a blank vial
- Always bake off prior to shutting down the system

Weekly Maintenance

- Check the liner and septa at least once a week (depending on frequency and number of injections)
  - Replace liner and septa if dirty

Bi-weekly Maintenance

- Every two weeks bake off column/oven, inlet, and detector for 1 hour (Inlet at 300° C; column/oven at 250°C; detector at 350°C, split ratio of 100:1.)

Monthly Maintenance

- Inlet Septa-check monthly; change if particulates are seen
- Inlet Liner-check monthly; look for ring left over from O-ring or dirt inside; change if dirty
- Inlet O-ring-change when change inlet liner

Annual/As Needed Maintenance

- Steam clean the HS: Run HS Method 4 using 10 reagent water vials containing 10 ml each
- Gold seal-every few months; look for particulates stuck to the top of the seal

    - If particulates are seen, replace the gold seal or clean with a cotton-swab and MeOH or soak in MeOH for 30 mins & rub, wearing gloves
- Jet-inspect and/or change jet if having detection issues, decreased sensitivity, noise, not lighting correctly (only check jet after other troubleshooting); jet is inside detector and must take side panel off GC to get to it

Column Maintenance and Storage

- Keep column capped whenever possible during maintenance etc.
- Always wear gloves when handling the column, column nuts, and ferrules
- Using smooth side of scoring wafer, make sure there is a clean, straight cut of the column before installing; put nut and ferrule on column before cutting
- Make sure the column is only 4-6mm above the ferrule/column nut on the inlet side
- Make sure the column is deadened against the jet and then pulled back slightly so that the column isn't resting on the top of the jet on the detector side
- Always install column in the same direction
- After installation, column should not touch inside walls of the oven
- When removing a column and replacing with a different column, immediately cap both ends before placing in the column box for storage
- When installing a new column, make sure to condition it at 20° C higher than the maximum method temperature for 1 hour
- Be aware of the column's maximum temperature listed by manufacturer

Standby Mode

- Upload Standby method in ChemStation
- Place blank vial in HS and adjust stop vial on HS
- Create a sequence table with 1 vial
- Run the sequence
    - Oven will bake off then system will stay in Standby mode
    - Turn off detector, inlet and oven after it cools below 35 C

CLEANING GLASSWARE

1. Clean all items used in laboratory sample preparation or analysis (i.e. glassware, utensils, bowls, etc) using a small amount of liquid Alconox detergent
    a. Prepare liquid Alconox as stated on the package
2. Rinse 3 times with reagent water
    a. If using the 3-compartment rinse sink, always rinse from front to back

DISPOSAL OF WASTE

1. Liquid waste shall be stored in liquid waste collection container
    a. Dispose liquid waste as needed at the City Solid Waste Facility
2. Cannabis samples remaining after testing is complete
    a. Log 3-day notice in METRC
    b. Hold remaining samples for 3 days
    c. Destroy samples by mixing in solid waste collection container with equal volume of paper waste or coffee grounds
        i. Log disposal in METRC
    d. Dispose destroyed solid was in trash
3. Biological waste (used microbial/media plates) shall be sterilized prior to disposal
    a. Store used media in the biohazard bin lined with an autoclave bag
    b. Autoclave the bag of used media at 121 degrees C for 20 minutes
    c. Dispose of sterilized bag in trash

STERILIZATION & STORAGE OF MICROBIOLOGY TOOLS

1. Place tools in autoclave
    a. Tools may be placed in an autoclave pouch or loose on the autoclave tray
        i. If loose, place a small length of autoclave indicator tape on 1 tool
2. Autoclave at 121 C for minimum 20 minutes
3. Observe sterilization indicators on pouches or tape to ensure proper sterilization

4. Loose items may be aseptically transferred to an enclosed, sterile storage container

EXTERNALLY PROVIDED PRODUCTS

Upon receipt, externally provided products shall be inspected to ensure they are in acceptable condition, documented in relevant log books (lot, batch, expiration) and stored in accordance with the manufacturer's recommendations. Applicable certifications shall be filed and retained in accordance with 3 AAC 306.755.

RECEIVING SAMPLES

1. Have the customer fill out the top, dark portion of the Chain of Custody (COC) form appropriate for each sample matrix delivered
    a. For State Licensed customers ensure that a Sampling State and Transport Manifest are received and documented on the COC
    b. Staff shall record their name in the "Received By" section
2. In the Sample Log-In book, record Date, Client Name, Description of Sample, Test(s) Requested and Weight received for each individual sample
    a. At this point each sample will receive a unique sample Identification Number (ID)
3. In the analysis section of the COC, record sample ID for each sample
    a. Assess whether the package and seal are in acceptable condition for each sample and record in the analysis section of the COC
4. Log the samples in METRC
5. Place the samples in storage containers labeled with sample IDs and store in the sample refrigerator until analysis
6. Store the COC in designated location on exterior of sample refrigerator until analysis

STORAGE REQUIREMENTS

- *Record batch #, lot #, date received and expiration date for all consumable products in appropriate log book(s)*
- *All solvents and chemicals shall be tightly sealed during storage*

DESCRIPTION	TEMP	LIGHT/DARK	LOCATION
Samples	Refrigerate	-	Refrigerator
Certified Reference Materials	Varies	Varies	As stated by manufacturer
Hardy Dry Plates (EC100, SL100, YMR100)	Room Temp	Dark	Storage Cabinet or Closet (in boxes)
Hardy Dilu-Lok Vials	Room Temp	Dark	Storage Cabinet or Closet (in boxes)
Hardy DRBC "Rose Bengal" Agar-Prepared	Refrigerate	-	Refrigerator
Buffered Peptone Water-Dry	Room Temp	-	Shelfs or Under Counter
Buffered Peptone Water-Prepared	Room Temp	-	Shelfs or Under Counter
Hardy EcoliPRO O157Kit	Refrigerate	No Direct Light	Refrigerator
Hardy MacConkey Agar	Refrigerate	No Direct Light	Refrigerator
UCT QuEChERS 2 ml DSPE Tubes	Room Temp	-	Storage Cabinet or Closet (in boxes)
UCT QuEChERS 5 g Pouches	Room Temp	-	Storage Cabinet or Closet (in boxes)
Acetic Acid	Refrigerate	-	Refrigerator
Formic Acid	Refrigerate	-	Refrigerator
Acetonitrile	Room Temp	-	Safety Storage Cabinet (Flammables)
Methanol	Room Temp	-	Safety Storage Cabinet (Flammables)
Isopropyl Alcohol	Room Temp	-	Safety Storage Cabinet (Flammables)
HPLC Water	Room Temp	No Direct Light	Safety Storage Cabinet (Flammables)
Dimethyl Sulfoxide (DMSO)	Room Temp	Dark	Storage Cabinet away from other open solvents and chemicals

CALIBRATIONS

HPLC – Bimonthly (twice per month) or per the Potency Analysis SOP

GC – Per Residual Solvents and Terpene SOPs

Precision balance – Annually by State of Alaska

Auto pipets – As needed based on performance testing every 6 months

Performance testing & calibration should be done using distilled water, room temperature between 15-30 C and humidity over 50%

Auto Pipets Performance Testing

1. Adjust to the desired test volume Vs
2. Carefully fit the tip onto the tip cone without twisting
3. Fill the tip with test water and expel to waste five times to reach a humidity equilibrium in the dead air volume
4. Replace the tip. Pre-wet the tip by filling it once with test water and expel to waste
5. Aspirate the test water, immersing the tip only 2-3 mm below the surface of the water keeping the pipette vertical
6. Withdraw the pipette vertically and touch the tip against the inside wall of the test water container
7. Pipette the water into the weighing vessel, touching the tip against the inside wall of the vessel just above the liquid surface at an angle of 30° to 45°. Withdraw the pipette by drawing the tip 8-10 mm along the inner wall of the weighing vessel
8. Record the weight
9. Repeat the test cycle until 10 measurements have been recorded
10. Convert the recorded masses (m_i) to volumes (V_i). $V_i = m_i * Z$,
    a. Z = correction factor (Table13)
11. Calculate the mean volume ($\bar{v}$) delivered:
    a. $\bar{v} = (\sum V_i)/10$
12. For conformity evaluation calculate the systematic error es of the measurement:
    a. in µl: $e_s = \bar{v} - V_s$, V_s = selected test volume
    b. or in %: $e_s = 100\ (\bar{v} - V_s)/V_s$

13. For conformity evaluation calculate the random error of the measurement:
    a. as standard deviation s = square root of $(\sum(V_i - \bar{v})^2) / (n-1)$
        i. n = number of measurement (10)
    b. or as coefficient of variation CV = $100s/\bar{v}$
14. Compare the systematic error (inaccuracy) and random error (imprecision) with the values in the performance specifications
    a. If the results fall within the specifications, the pipette is ready for use. Otherwise check both systematic and random errors and, when necessary, proceed to the recalibration procedure

Table 13

Z-values (µl/mg):

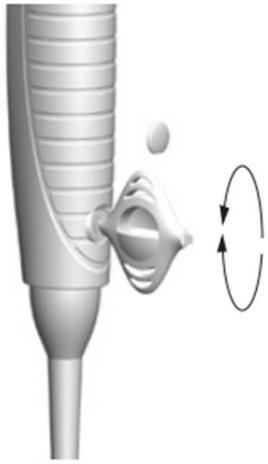
Temp. (°C)	Air Pressure (kPa)			
	95	100	101.3	105
20.0	1.0028	1.0028	1.0029	1.0029
20.5	1.0029	1.0029	1.0030	1.0030
21.0	1.0030	1.0031	1.0031	1.0031
21.5	1.0031	1.0032	1.0032	1.0032
22.0	1.0032	1.0033	1.0033	1.0033
22.5	1.0033	1.0034	1.0034	1.0034
23.0	1.0034	1.0035	1.0035	1.0036
23.5	1.0036	1.0036	1.0036	1.0037

NOTE: This method is based on ISO 8655.

Auto Pipets Recalibrate

1. Remove the lid of the calibration nut, located on the back of the handle, with the aid of the calibration tool (see graphic below)
2. Place the hexagonal head of the calibration tool into the hole of the calibration nut
3. Turn the adjustment lock counterclockwise to decrease and clockwise to increase the volume
4. Repeat testing the performance procedure. Continue until the results are correct

NOTE: Recalibration is recommended if reverse pipetting technique is used



Analytical balance – Weekly (minimum)

1. Clean the balance pan
2. Press the “Cal” key. The display will show “Load 0”
3. Press the “Set Up” key. After a moment the display with show “Load 50”
4. Place the 50 g calibration mass on the pan using tweezers. The balance will automatically continue
5. After a beep sounds the display will show “Unload”
6. Remove the mass back to its storage container
7. A final beep indicates that calibration is complete and the balance is ready for use