

North Coast Testing Laboratories, LLC		Standard Operating Procedure	
Title: Determination of Select Cannabinoids in Cannabis Flower and Processed Products by HPLC			Effective Date: April 23, 2025
SOP No: SOP-140	Rev No: 06	Supersedes: Rev. 05	Page 1 of 23

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Date: See QT9 E-Sign

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Date: See QT9 E-Sign

Technical Approval: Ryan Randolph, Director of Science

Date: See QT9 E-Sign

## 1 SCOPE AND APPLICATION

- 1.1 This SOP describes the procedure for determination of select cannabinoids in cannabis flower, concentrates, and cannabis-infused products by high performance liquid chromatography (HPLC).
- 1.2 This SOP is applicable to cannabinoid determination in cannabis flower, concentrated extracts of marijuana and hemp flowers, as well as edible and topical products that have been infused with the same. See each specific SOP for preparation procedures for each sample type.
- 1.3 Specific cannabinoids determined by this analytical method include the naturally occurring acidic forms (CBDA, CBGA, THCA, and THCVA) as well as the decarboxylated forms (CBD, CBDV, CBG, CBN, Δ8-THC, Δ9-THC, Δ9-THCV, Δ8-THCV and CBC).

## 2 HEALTH AND SAFETY WARNINGS

- 2.1 HPLC systems are intended for use by qualified and trained personnel. HPLC systems must not be used until the analyst has read and familiarized themselves with the instructions contained in the user manual.
- 2.2 The analyst must be familiar with the appropriate PPE such as safety glasses and gloves when working with chemicals or near equipment. Users of this procedure must be cognizant of inherent laboratory hazards.
- 2.3 All laboratory personnel performing this analysis should be familiar with the SDS for all materials used in this procedure.
- 2.4 Proper PPE, such as gloves and lab coat, are required when working in the laboratory.
- 2.5 Use of an open flame in the vicinity of an HPLC instrument is strictly prohibited.
- 2.6 Follow the approved Chemical Hygiene Plan (CHP), SOP-302. This includes awareness of the proper disposal procedures of contaminated materials and appropriate segregation of hazardous wastes.

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### 3 DEFINITIONS AND ABBREVIATIONS

- 3.1 Analysis Batch – A group of samples analyzed by HPLC on the same day, by the same analyst, using the same calibration curve and mobile phase lots.
- 3.2 MDL (Method Detection Limit) – As defined by 40 CFR Part 136, it is the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results.
- 3.3 LLOQ (Lower Limit of Quantitation) – This is the reporting limit that is the lowest concentration that can be accurately quantified by this method.
- 3.4 ULOQ (Upper Limit of Quantitation) – This is the maximum concentration that can be accurately quantified by this method.
- 3.5 QS (Quantity Sufficient) – To bring the level of solution up to final volume with the bottom of the meniscus touching the top of the final volume line.
- 3.6 Sample Preparation Batch – A group of samples, consisting of a single matrix type (e.g. cannabis flower, concentrates, infused topicals, or infused edibles) which are prepared for analysis using a single preparation procedure, on a single day, by a single analyst using the same reagents and laboratory equipment. Multiple sample preparation batches may be combined into a single Analysis Batch.

#### 3.7 Abbreviations

CBC	Cannabichromene
CBD	Cannabidiol
CBDA	Cannabidiolic Acid
CBDV	Cannabidivarin
CBG	Cannabigerol
CBGA	Cannabigerolic Acid
CBN	Cannabidiol
$\Delta$ 8-THC	Delta-8-tetrahydrocannabinol
$\Delta$ 9-THC	Delta-9-tetrahydrocannabinol
THCA	Delta-9- tetrahydrocannabinolic acid
THCV	Delta-9- tetrahydrocannabivarin
$\Delta$ 8-THCV	Delta-8- tetrahydrocannabivarin
THCVA	Delta-9-tetrahydrocannabivarinic acid
HPLC	High Performance Liquid Chromatography

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PDA	Photodiode Array
mL	Mililiter
P/N	Part Number
µL	Microliter
ISO	International Organization of Standardization
NCTL	North Coast Testing Laboratories
PPE	Personal Protective Equipment
SDS	Safety Data Sheet
SOP	Standard Operating Procedure
ICB	Initial Calibration Blank
ICV	Initial Calibration Verification
LCS	Laboratory Control Sample

## 4 REAGENTS, MATERIALS AND EQUIPMENT

### 4.1 Instrumentation

- 4.1.1 Shimadzu u Prominence-i LC-2030C Plus, Nexera UHPLC, or equivalent, with PDA detector, or equivalent.
- 4.1.2 LabSolutions® Operating Software

### 4.2 Instrument Consumables

- 4.2.1 Shimadzu NexLeaf CBX or Restek Raptor ARC-18, 2.7 µm, 4.6 x 150 mm Column (Shimadzu P/N: 220-91525-70 or Restek P/N: 9314A65)
- 4.2.2 Shimadzu NexLeaf CBX or Restek Raptor ARC-18 EXP Guard Cartridge, 2.7 µm, 4.6 x 5 mm, 3/pkg (Shimadzu P/N: 220-91525-72, or Restek P/N: 9314A0250)
- 4.2.3 Shimadzu NexLeaf Guard Cartridge Holder (Shimadzu P/N: 220-91525-73 or Restek P/N: 25808)

### 4.3 Laboratory Equipment and Glassware

- 4.3.1 Dispensette S Organic Bottletop Dispenser, Digital, 1-10 mL, BrandTech Scientific, Inc. P/N: 4630340, or equivalent.
- 4.3.2 Dispensette S Organic Bottletop Dispenser, Digital, 5-50 mL, BrandTech Scientific, Inc. P/N: 4630360, or equivalent.
- 4.3.3 Calibrated 5 mL air-displacement pipette (500-5000 µL )

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- 4.3.4 Fluid Management 5G Mixer
- 4.3.5 Geno Grinder – SPEX Sample Prep 2010 or equivalent
- 4.3.6 Analytical Balance - Shimadzu AUW220D or equivalent
- 4.3.7 Centrifuge – Thermo Scientific Sorvall ST40 R or equivalent
- 4.3.8 Vortex Mixer – SciloGex MX-S or equivalent
- 4.3.9 Calibrated repeater pipette, Eppendorf Model M4, P/N: 4982000322, or equivalent.
- 4.3.10 Ultrasonic Bath – Fisher Scientific CPX-8800, or equivalent
- 4.3.11 Class A Graduated Cylinders, various sizes
- 4.3.12 Class A Volumetric Flask, various sizes
- 4.3.13 Test Sieve, 4.75 mm (4 Mesh), ASTM E-11, Fisherbrand No. 4, Fisher Scientific P/N: 04-881-10B, or equivalent.
- 4.3.14 Test Sieve, 2.36 mm (8 Mesh), ASTM E-11, Fisherbrand No. 8, Fisher Scientific P/N: 04-881-10F, or equivalent.
- 4.3.15 Test Sieve, 1.18 mm (14 Mesh), ASTM E-11, Fisherbrand No. 16, Fisher Scientific P/N: 04-881-10K, or equivalent.
- 4.3.16 Test Sieve, 600  $\mu$ m (28 Mesh), ASTM E-11, Fisherbrand No. 30, Fisher Scientific P/N: 04-881-10P, or equivalent.
- 4.3.17 Calibrated Hamilton Gas Tight Syringes, 50  $\mu$ L (Part# CAL80975), 100  $\mu$ L (Part# CAL81065) and 1 mL (Part# CAL81317), or equivalent
- 4.4 Laboratory Consumables
  - 4.4.1 50 mL Centrifuge Tubes
  - 4.4.2 Pipette tips, 5 mL
  - 4.4.3 Eppendorf Combitips, 5 mL, P/N: EP0030089456, or equivalent
  - 4.4.4 Autosampler 2.0 mL, 9mm short-cap, screw-thread vials, Restek P/N: 21143, or equivalent
  - 4.4.5 2.0 mL, 9 mm Short-Cap, Screw-Vial Closures, Restek P/N: 24486, or equivalent
  - 4.4.6 2 mL screw cap vial with PTFE/rubber septum.
  - 4.4.7 Low-volume inserts for 2 mL vials, 250  $\mu$ L, Restek P/N: 21776, or equivalent
  - 4.4.8 Parafilm – Laboratory sealing film
  - 4.4.9 5.55 mL Borosilicate glass vial with Phenolic Screw Cap, VWR P/N 66011-063, or equivalent.
  - 4.4.10 16 oz Sample Jar – Weber Scientific PN. 3041-92, or equivalent

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## 5 REAGENTS AND STANDARDS

- 5.1 Phosphoric acid, ACS reagent grade, 85+% solution in water; Acros Organics #44204 or equivalent
- 5.2 Isopropanol, HPLC Grade; Honeywell #34965 or equivalent
- 5.3 Water, HPLC grade; Fisher Scientific Optima #W6-4 or equivalent
- 5.4 Acetonitrile, HPLC grade; Fisher Scientific Optima #A955 or equivalent
- 5.5 Methanol, HPLC grade; Fisher Scientific Optima #456 or equivalent
- 5.6 250 µg/mL Eleven Cannabinoids Standard Mixture, Shimadzu #220-91239-21 or equivalent
- 5.7 Cannabinoid Mixture (Neutral) – 8 components, Cerilliant #C-219 or equivalent.
- 5.8 Cannabinoid Mixture (Acids) – 6 components, Cerilliant #C-218 or equivalent.
- 5.9 1 mg/mL THCVA (CRM), Cerilliant # T-111 or equivalent.
- 5.10 1 mg/mL d8-THCV (CRM), Cerilliant # T-175 or equivalent.
- 5.11 QuEChERS Salt packets – Restek #25849 or equivalent

## 6 PREPARATION OF REAGENTS, SOLUTIONS AND STANDARDS

- 6.1 **Mobile Phases:** Record mobile phase preparations in the Reagent Logbook. Record standard preparations in the Standard Logbook.
  - 6.1.1 Mobile Phase A – Using a 5-mL pipette, transfer 4.0 mL of 85% phosphoric acid directly into a newly acquired 4-L jug of HPLC grade water and label with its respective solution ID that is recorded into HPLC reagents logbook. Mix thoroughly.
  - 6.1.2 Mobile Phase B – Using a 5-mL pipette, transfer 4.0 mL of 85% phosphoric acid directly into a newly acquired 4-L jug of HPLC grade acetonitrile and label with its respective solution ID that is recorded into HPLC reagents logbook. Mix thoroughly.
- 6.2 **Calibration Standards:** Prepare an 8-point calibration curve with a range of 0.25 µg/mL to 250 µg/mL using the 11-part cannabinoid CRM, or equivalent from another vendor. *The procedure below uses a stock standard mixture containing each analyte at 250 µg/mL.* Comparable calibration standards from other vendors or at different concentrations may be exchanged, provided the concentrations of analytes are appropriately accounted for.
  - 6.2.1 Retrieve an unopened ampule of the Cayman 11 part cannabinoid CRM from frozen storage and equilibrate to ambient temperature. Vortex mix before opening.
  - 6.2.2 Snap the glass vial at the score line and transfer the contents using a Pasteur pipet into a 2 mL screw cap vial with PTFE/rubber septum and label as 250 µg/mL master mix with the lot number and today's date.

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6.2.3 Calibration standards must be prepared with the 1 mL glass syringe.

6.2.4 Prepare each calibration standard using the values given in the tables below:

11-part cannabinoid calibration standards				
Parent Solution (µg/mL)	Aliquot Volume (µL)	MeOH Volume (µL)	Total Volume (µL)	Final Concentration (µg/mL)
250 (CRM)	1000	0	1000	250
250 (CRM)	400	600	1000	100
100	500	500	1000	50.0
50.0	200	800	1000	10.0
10.0	500	500	1000	5.00
5.00	200	800	1000	1.00
1.00	500	500	1000	0.500
0.500	500	500	1000	0.250

6.2.5 Transfer approximately 60 µL of each standard to a separate vial with a low volume insert for HPLC calibration.

6.2.6 Seal primary vials, containing calibration standards, with Parafilm and store at at ≤20 °C for up to three months from date of preparation.

6.3 **Independent Calibration Verification (ICV, 100 µg/mL)** – Using a 1 mL gas tight syringe, add 3000 µL of methanol, 1000 µL of the Cerilliant 6 component Cannabinoid mixture (C-218), and 1000 µL of the Cerilliant 8 component Cannabinoid mixture (C-219) reference standards to a 5.55 mL screw cap vial with a phenolic screw cap. Cap, vortex, and label accordingly. This will be the working standard ICV concentration of 100 µg/mL. *Seal with Parafilm and store at ≤ -20°C for up to three months.* This can also be used as an alternative CCV. The CCV cannot be used as an ICV.

6.4 **Continuing Calibration Validation Preparation (CCV, 100 µg/mL)** – In a 5.55 mL screw cap vial with a phenolic screw cap, add 1500 µL methanol followed by 1000 µL of the 250 µg/mL Cayman Eleven Cannabinoids Standard Mixture using a 1mL gas tight syringe. Cap and vortex. *Store at ≤ -20 °C for up to three months.*

6.5 **d8-THCV (100 µg/mL)** – In a 2 mL HPLC vial, add 900 µL methanol with a 1 mL gas tight syringe, followed by 100 µL of Cerilliant d8-THCV CRM standard (T-175). Cap and vortex. *Store at ≤ -20 °C for up to three months.*

6.6 **Reporting Limit Check (RLC, 0.25 µg/mL)** – In an HPLC vial with glass insert, add 50 µL of the 0.25 µg/mL 11 mix standard.

6.7 **High Limit Check (HLC)** – In an HPLC vial with glass insert, add 50 µL of the 250 µg/mL 11 mix standard.

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## 6.8 Laboratory Control Sample (LCS)

### 6.8.1 Flower LCS:

- 6.8.1.1 The LCS is a bulk sample of homogenized cannabis flower that is sampled and analyzed once with each batch and control charted over time to monitor stability of method performance.
- 6.8.1.2 Select a variety dried cannabis flower test samples that are eligible for disposal. Samples with diverse cannabinoid profiles should be selected to maximize the number of analytes with quantifiable results.
- 6.8.1.3 A sufficient quantity of cannabis flower material should be obtained to allow use of a single lot of LCS for at least one year. Typically, at least 100 g is sufficient, but 500 g or more is recommended.
- 6.8.1.4 Homogenize all selected samples in a food processor, or similar device, until material is reduced to a powder.
- 6.8.1.5 Stack the sieves in order of increasing Mesh from top to bottom (i.e. 4 > 8 > 14 > 28 Mesh). Pass homogenized flower material through the stack of sieves. Discard material that does not pass through the entire stack of sieves.
- 6.8.1.6 Collect all sieved material in a large vessel. Thoroughly mix the material to ensure homogenous distribution of cannabinoids throughout the entire batch.
- 6.8.1.7 Distribute the fully homogenized material into separate glass jars, placing approximately 10 to 20 g into each jar.
- 6.8.1.8 Assign the entire LCS batch a unique lot number in the format "yymmdd". Label each jar with the lot number followed by a suffix indicating the "n<sup>th</sup>" jar out of the total number of jars (e.g. "LCS-220420-1/20"). Cap the jars and seal with parafilm. Store at  $\leq -15^{\circ}\text{C}$  when not in use.
- 6.8.1.9 It is recommended to analyze one aliquot from each jar to verify homogenous distribution across all jars. All quantifiable cannabinoids should be within  $\pm 3$  standard deviations from the mean to be considered acceptable.
- 6.8.1.10 Adjust the weights of each package in METRC according to how much was taken to prepare the LCS sample and then create a new package ID for the LCS.

### 6.8.2 Processed Product LCS

- 6.8.2.1 The Processed Product LCS is a bulk sample of homogenized distillate concentrate that is sampled and analyzed once with each prep batch and control charted over time to monitor stability of method performance.
- 6.8.2.2 Select a variety of concentrate distillate test samples that are eligible for disposal. Samples with diverse cannabinoid profiles should be selected to maximize the number of analytes with quantifiable results.

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- 6.8.2.3 A sufficient quantity of concentrate distillate material should be obtained to allow use of a single lot of LCS for at least one year. Typically, at least 500 g is sufficient, but 500 g or more is recommended.
- 6.8.2.4 Melt all selected samples in 60C water bath and combine into one container. Mix thoroughly to obtain a fully homogenized product.
- 6.8.2.5 Distribute the fully homogenized material into separate glass jars, placing approximately 10 to 20 g into each jar.
- 6.8.2.6 Assign the entire LCS batch a unique lot number in the format “yymmdd”. Label each jar with the lot number followed by a suffix indicating the “n<sup>th</sup>” jar out of the total number of jars (e.g. “LCS-220420-1/20”). Cap the jars and seal with parafilm. Store at  $\leq -15^{\circ}\text{C}$  when not in use.
- 6.8.2.7 It is recommended to analyze one aliquot from each jar to verify homogenous distribution across all jars. All quantifiable cannabinoids should be within  $\pm 3$  standard deviations from the mean to be considered acceptable.
- 6.8.2.8 Adjust the weights of each package in METRC according to how much was taken to prepare the LCS sample and then create a new package ID for the LCS.

## 7 RECEIVING, PREPARATION, AND STORAGE

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- 7.1 Refer to SOP-232, Sample Receiving, NCTL.
- 7.2 Refer to SOP-233, Sample Storage Procedure, NCTL.
- 7.3 Refer to SOP-234, Cannabis Waste, NCTL.
- 7.4 Aliquots for cannabinoid potency analysis shall be taken from the sample jar only after aliquots for microbial contaminant analysis have been collected. If two jars of the same sample are provided, then one jar may be designated for all non-microbial testing.

## 8 CALIBRATION AND STANDARDIZATION OF HPLC

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- 8.1 Refer to LabSolutions user manual for instructions on how to create a batch file, initiate data acquisition and create a calibration table.
- 8.2 Create a new folder within the LabSolutions data folder directory. Name the folder in a manner that references the calibration date followed by the word ‘calibration’ (e.g. 220420\_calibration).
- 8.3 Make a copy of a calibration template batch file, rename it with today’s date, and place it in the newly created folder.
- 8.4 When the batch has been completed, use Post-run Analysis or QuantBrowser software to review calibration results.



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## 9 QUALITY CONTROL AND ASSURANCE

- 9.1 Reference standards shall not be used beyond their expiration dates.
- 9.2 All laboratory equipment used to measure sample mass, extraction volume, or aliquot volume for dilution must be verified for accuracy on each day of use.
- 9.3 **Calibration Curve**
  - 9.3.1 The calibration curve for all compounds must have a correlation coefficient of  $\geq 0.995$ . Any curve that is  $< 0.995$  must be rerun.
  - 9.3.2 The calibration curve must meet acceptance criteria established in the Cannabinoid Potency Calibration QC Template.
- 9.4 **Independent Calibration Verification (ICV)** - The ICV is a quality control standard that is run immediately following calibration to ensure accuracy against an independent standard. This standard must be from a separate source than that used to make the calibration standards.
  - 9.4.1 The ICV only needs to be run when the instrument is calibrated.
  - 9.4.2 The ICV results must be within  $\pm 10\%$  of the expected values.
    - 9.4.2.1 If the ICV results differ by more than  $\pm 10\%$  from the expected values, remake and rerun the ICV once.
    - 9.4.2.2 If the second ICV results are within  $\pm 10\%$  of expected values then the run may continue. Otherwise, the instrument must be recalibrated.
- 9.5 **d8-THCV** – The d8-THCV standard is a quality control standard used to run in conjunction with products infused with THC isomers, such as gummies. It is used to ensure that the surrogate analyte in the calibration, d9-THCV, is properly qualifying d8-THCV and has the correct retention time. This can be run immediately after a calibration to establish an accurate correction factor for the surrogate analyte, d9-THCV.
  - 9.5.1 The d8-THCV recovery limits are  $\pm 10\%$  of the expected values.
    - 9.5.1.1 If the d8-THCV is outside the control limits, re-prepare and rerun a new d8-THCV standard.
    - 9.5.1.2 If the second d8-THCV standard passes, then the run may continue. If it is still outside of the control limits, then the instrument must be recalibrated.
- 9.6 **Reporting Limit Check (RLC)** – The RLC is a quality control standard run at a concentration equal to the LLOQ for all compounds. The RLC is used to monitor the sensitivity of the instrument but does not need to be run daily or as part of the regular batch QC.
  - 9.6.1 The RLC recovery limits are  $\pm 50\%$  of the expected values.
    - 9.6.1.1 If the RLC is outside the control limits, reprepare and rerun a new RLC.

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9.6.1.2 If the second RLC passes, then the run may continue. If it is still outside of the control limits, then the instrument must be recalibrated.

9.7 **High Level Check (HLC)** – The HLC is a quality control standard run at the ULOQ point of the calibration curve. The HLC is used to monitor the calibration curve's accuracy at the upper portion of the curve, but does not need to be run daily or as part of the regular batch QC.

9.7.1 The HLC recovery limits are +/- 10% of the expected values.

9.7.1.1 If the HLC is outside the control limits, reprep and rerun a new HLC.

9.7.1.2 If the second HLC passes, then the run may continue. If it is still outside of the control limits, then the instrument must be recalibrated.

9.8 **Continuing Calibration Verification (CCV)** - The CCV is a quality control standard run at the beginning of each analytical run, after every 20 samples, and at the end of the run to verify continuous accuracy of the current calibration.

9.8.1 The CCV results must be within  $\pm 10\%$  of the expected values.

9.8.1.1 If the CCV results differ by more than  $\pm 10\%$  of the expected value, remake and rerun the CCV once.

9.8.1.2 If the second CCV results are within  $\pm 10\%$  of the expected values, then the run may continue. Otherwise, the instrument must be recalibrated and all samples before and after the failing CCV must be re-analyzed.

9.8.1.3 The ICV may be used as an alternative to the CCV and must fit the same criteria that applies to the CCV.

9.9 **Continuing Calibration Blank (CCB)** - The CCB is a negative control that is run at the beginning of each analytical run, after every 20 samples, and at the end of the run. The CCB verifies the continuous cleanliness of the instrument as well as the stability of the baseline signal.

9.9.1 The CCB must be <LOQ for all compounds.

9.9.1.1 If the CCB is greater than the LOQ, pour a fresh blank and rerun the CCB once.

9.9.1.2 If the second CCB passes, then the run may continue. If it is still greater than the LOQ, then the instrument may need cleaned and all samples before and after the failing CCB must be re-analyzed.

9.10 **Laboratory Control Blank (LCB)** – THE LCB is a negative control that is prepared with each preparation batch. The LCB verifies that no contamination occurred during the preparation of samples.

9.10.1 The LCB is prepared along with the samples using the same extraction procedure. The result of the LCB must be < LOQ. If the LCB result > LOQ, check to see if the contaminating analyte is less than 10 times the value of the lowest reporting value in the samples. If this is the case, the samples can be reported. If this is not the case, then consult with quality manager, laboratory director or scientific director.

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9.10.2 Alternatively, the acquired data can be reported if all analytes quantify above the new instrument quantification limit calculated by the quality manager, laboratory director or scientific director.

9.11 **The Laboratory Control Sample (LCS)** is prepared once concurrently with each preparation batch of test samples. For processed products, there is a concentrate LCS that is prepared only with concentrate samples.

9.11.1 LCS results for Total THC ( $\Delta 9\text{-THC} + \Delta 8\text{-THC} + 0.877 \times \text{THCA}$ ) should be within  $\pm 3$  standard deviations of the mean value. If LCS results are outside this range, associated test sample results should not be reported until the analyst is able to confirm that the source of error did not impact test sample results.

9.11.2 If a failing LCS result occurs, the analyst may re-prepare and/or re-dilute and re-analyze the LCS extract concurrently with at least 3 random test samples from the same batch. If the reanalyzed LCS passes, and Total THC measured in the 3 test samples is within  $\pm 10\%$  relative to the original result, then the original batch results may be reported.

9.12 **Samples** - Test sample results which are above the top calibration standard (250  $\mu\text{g/mL}$ ) must be diluted sufficiently enough to bring it within the dynamic range, but not more than necessary, to avoid low concentration analytes from falling below the LLOQ.

9.13 **Initial Demonstration of Capability (IDOC)** - Each analyst must demonstrate initial proficiency by generating data of acceptable accuracy and precision for a minimum of four replicates of a reference sample containing all eleven cannabinoids in a clean matrix and have four analytical batches approved under secondary validation review.

9.14 **On-Going Demonstration of Capability (DOC)** - Each analyst must demonstrate proficiency in the analytical techniques performed annually. Performance is demonstrated by analyzing a minimum of four replicates of a reference sample containing all eleven cannabinoids in a clean matrix. The average recovery and standard deviation obtained must meet criteria for the analyst to be able to continue performing this analysis.

#### 9.15 Control Chart

9.15.1 Control chart is monitored for each analyte for the LCS.

9.15.2 The control chart limits are established continuously, and acceptance limits are updated on an annual basis.

#### 9.16 Documentation

9.16.1 Data Validation Sheet – This is an excel file that resides with the data on the shared drive. It contains information regarding calibration information, QC, reagent IDs, standard IDs, and expiration dates. This excel spreadsheet must be filled out with every batch of samples.

9.16.2 Standard Log – This is a log for all prepared standards. It contains information about volumes and expiration dates. Every standard that is prepared must be put into the log and given a unique identifier.

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9.16.3 Reagent Log - This is a log for all prepared reagents. It contains information about volumes and expiration dates. Every reagent that is prepared must be put into the log and given a unique identifier.

9.16.4 Sample Preparation Log – This is the Sample Preparation Log for all samples. Weights and volumes are recorded for all samples as well as any dilutions that are made. Reagents that are used in the extraction process are documented on the form.

## 10 PROCEDURE

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10.1 **Sample Preparation:** Refer to SOPs 125, 126, 127, 128, 129, 130, 132, 133, and 146 depending on the sample type. Form DOC-120 summarizes the above.

### 10.2 HPLC Analysis:

10.2.1 Refer to Appendix A for specific acquisition method parameters.

10.2.2 Refer to the LabSolutions software user manual for instructions on how to create a batch file and initiate data acquisition.

10.2.3 Injection volume = 5 µL.

10.2.4 Ensure the batch file includes columns labelled “Weight (G)”, “Volume (L)”, “Rep. Number”, “Dilution”, and “Analyst”. Populate the cells in these columns as follows:

- Weight (G) = sample weight from Prep Batch bench sheet
- Volume (L) = extraction volume in liters from Prep Batch bench sheet (e.g. 20 mL = 0.02 L)
- Dilution = dilution factor from Prep Batch bench sheet, typically 20 for flower.
- Rep. Number = 1 (LIMS currently requires this field but does not parse it to assign a rep number to each data file.)
- Analyst = First and last initials of analyst performing HPLC analysis (e.g. John Doe = JD)

10.2.5 Method File: Select the method containing the most recent acceptable calibration data. The method filename should include a date code referencing the date of calibration (e.g. “220420\_HighSensitivity.lcm”).

10.2.6 Raw data files should be acquired to a directory location within the LabSolutions data folder that corresponds the year, month, and date of data acquisition: (e.g. “C:\LabSolutions\Data\2022\04-April\220420”).

### 10.3 Data Processing

10.3.1 Verify that all CCV, CCB, LCB, and LCS results are within specifications listed in Section 9. Verify that all the retention times are correct for all QC and unknown samples and that all peak shapes are typical.

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10.3.2 Address any non-conforming QC results using DOC-106. Enter all QC results data into the corresponding control charts and batch validation sheets.

10.3.3 If results are acceptable for reporting, transfer raw data to LIMS as follows:

- Select all samples to be reported in the Data Navigation pane of the Post-run Analysis or QuantBrowser software.
- Right click on the selected samples, select “File Conversion” then “Convert LabSolutions Data file to ASCII File”
- Select “Output Items...” and confirm that “Data File Properties”, “Peak Table” and “Identified Result” options are selected. Save the ASCII files in the same folder as the data acquisition files.

10.3.4 Copy the ASCII files to the following folder: “S:\NorthCoast\data\ShimHplc”. The LIMS system will automatically sweep data files from this location and upload results into LIMS.

10.3.5 Use an internet browser to access the LIMS system at <https://lims5000.azurewebsites.net/>

10.3.6 Navigate to the Batch Workflow screen and filter for the current analytical batch to be reviewed.

10.3.7 Select “Batch Results” to review results.

10.3.8 Each raw data text file that has been uploaded to LIMS will appear as a separate replicate when the “+” sign next to each analyte is clicked.

10.3.8.1 Results from all included replicates will be averaged for the reported result.

10.3.8.2 Exclude any invalid replicates. Enter the reason for exclusion when prompted. See specific policy/procedure for flower and concentrate confirmations.

10.3.8.3 The Grubb’s test may be used to identify statistical outliers at 95% confidence interval ( $\alpha = 0.05$ ). A simple calculator for performing the Grubb’s test is available at: <https://www.graphpad.com/quickcalcs/grubbs1/>

10.3.9 Once results in LIMS have been reviewed and deemed acceptable for reporting, use the “Set to Complete” button to indicate an individual sample is ready to report, or “Set All Tests to Complete” button to indicate all samples within a given analytical batch are ready to report.

#### 10.4 Normalization of Results >100% Total Cannabinoids

10.4.1 Total cannabinoid results >100% may occur due to random and systematic error and normal variability inherent to all analytical determinations.

10.4.2 Since it is physically impossible for a true concentration to be greater than 100%, all cannabinoid potency values for any sample where total cannabinoids measured is >100% should be normalized to 100% total cannabinoids by adjusting all individual cannabinoid results proportionally.

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- 10.4.3 Normalized results must be flagged on the raw data and documentation listed so that the customer will be informed of this adjustment on the final report. Document normalization on the data validation sheet.
- 10.4.4 Enter the following note into the LIMS so that it transfers to the final report: "Original analytical results yielded a total cannabinoid value of XXX%. Reported results have been normalized to a total cannabinoid value of 100%", where "XXX%" represents the original total cannabinoid value prior to normalization.

## 11 CONFIRMATIONS

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- 11.1 Confirmations may be run for a variety of reasons. These reasons include but are not limited to:
- 11.1.1 The result is outside the customer's target range.
  - 11.1.2 The result does not meet the conditions outlined in Section 12 and 13.
  - 11.1.3 The customer requests a confirmation
- 11.2 If the original analysis was conducted as a single replicate, then the confirmation analysis may be conducted in duplicate. If the original analysis was conducted in duplicate, then the confirmation analysis may be conducted in triplicate. If the original analysis was conducted in triplicate, then the confirmations analysis may be conducted in duplicate.
- 11.3 Review the resulting data for outliers using the Grubb's test, if applicable.  
If a single point is qualified as an outlier by the Grubb's test, then exclude it and report the average of the remaining replicates. Please refer to sections 12 and 13 for additional exclusion policies.

## 12 SPECIFIC POLICY/PROCEDURE FOR FLOWER SAMPLE CONFIRMATION

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- 12.1 **When there are less than 20 historical data points for any number of samples of a variety:** If the historical average is less than 19.9% total THC, then results that are +/- 3% or greater from the historical average may be confirmed. If the historical average is greater than 20.0%, then confirmations may be performed when results are +/- 4% or greater from the historical average.
- 12.2 **When there is greater than 20 historical data points:** If the result is greater than +/- 2 standard deviations from the historical average, then confirmations may be run.
- 12.3 **One Sample of a Variety from a Harvest:** If the results meet the conditions set forth in 12.1 or 12.2, then a confirmation analysis may be performed. Additionally, if any result is not qualified as an outlier by the Grubb's test and the difference between the putative outlier(s) and mean of the remaining replicates is greater than 3.5 percentage points total THC, then the suspected outlier(s) may be excluded.

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- 12.4 **Two Samples of a Variety from a Harvest:** If any of the results meet conditions set forth in 12.1 and 12.2, then confirmations on that sample may be performed. Additionally, if the sample's results are greater than 3.5% total THC from each other, then confirmations may be run on both samples.
- 12.5 **Three or More Samples of a Variety from a Harvest:** If any of the results meet conditions set forth in 12.1 and 12.2, then confirmations on that sample may be performed. Additionally, if any one of the sample's results are greater than 3.5% total THC from average of the others, then confirmations may be ran on that sample.

## 13 SPECIFIC POLICY/PROCEDURE ON CONFIRMATION OF CONCENTRATES SAMPLES

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- 13.1 If total THC in a distillate measures >93% on the first pass, then confirmations on the sample may be run.
- 13.2 If the total THC result obtained is more than 3.5 percentage points above or below an established historical average, then confirmations may be ran as listed below:
- 13.2.1 If the original analysis was conducted in duplicate, then the confirmation may be conducted in triplicate (5 total).
- 13.2.2 If the original analysis was conducted in triplicate, then the confirmation may be conducted in duplicate (5 total).
- 13.2.3 If the single point is not qualified as an outlier by the Grubb's test, but the difference between the putative outlier and mean of the remaining replicates is greater than 3.5 percentage points total THC, then the suspected outlier may be excluded.

## 14 CALCULATIONS

---

- 14.1 Accuracy % of the Standard Curves  
Accuracy % =  $C_m / C_r \times 100$

where:

$C_m$ : Measured concentration

$C_r$ : Expected (theoretical) concentration

- 14.2 The following calculations are performed automatically in LIMS:

- 14.2.1 For processed products (concentrates and infused products), results are reported in mg/g.

$$\text{Result (mg/g)} = C_i \times DF \times EV \div SW \times 1 \text{ mg/1000 } \mu\text{g}$$

Where:

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$C_i$  = Concentration from the instrument ( $\mu\text{g/mL}$ )

DF = Dilution Factor

EV = Extraction Volume (mL)

SW = Sample weight (g)

14.2.2 For flower, results are reported as a % by weight on an “as-is” basis:.

$$\text{Result (\%)} = C_i \times DF \times EV \div SW \times 1 \text{ g}/10^6 \mu\text{g} \times 100\%$$

Where:

$C_i$  = Concentration from the instrument ( $\mu\text{g/mL}$ )

DF = Dilution Factor

EV = Extraction Volume (mL)

SW = Sample weight (g)

14.3 Total THC = THCA  $\times$  0.877 +  $\Delta$ 9-THC +  $\Delta$ 8-THC

Where:

[THCA] = Concentration of THCA

[ $\Delta$ 9-THC] = Concentration of  $\Delta$ 9-THC

[ $\Delta$ 8-THC] = Concentration of  $\Delta$ 8-THC

0.877 = molecular weight conversion factor ( $\Delta$ 9-THC (g/mol) / THCA (g/mol))

14.4 Total CBD = [CBDA]  $\times$  0.877 + CBD

Where:

[CBDA] = Concentration of CBDA

[CBD] = Concentration of CBD

0.877 = molecular weight conversion factor ( $\Delta$ 9-THC (g/mol) / THCA (g/mol))

14.5 Relative Response Factor for determining concentration of THCVA

14.5.1 THCVA uses a relative response factor for calculating concentration and is determined by the ratio of the peak area of  $\Delta$ 9-THCV to the peak area of THCVA from the CRM Cerilliant Standards, C-218 and C-219. This ratio is then applied as a correction factor in the method file using the calibration curve of  $\Delta$ 9-THCV.

Where:

$$\text{RRF} = \text{Peak Area } \Delta 9\text{-THCV} / \text{Peak Area } \text{THCVA}$$

14.6 Calculation for Normalization of the Cannabinoids Concentration

14.6.1 Use the following equation to calculate the normalized concentration (%) of each cannabinoid,  $C_n$ , from the measured concentration (%),  $C_m$ , and the measured total cannabinoid concentration (%),  $TC_m$ :



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$$C_n = \frac{C_m}{TC_m} \times 100\%$$

Cannabinoid	Original Results (%, w/w)	Normalized Results (%, w/w)*
CBDV	0	0
CBDA	0	0
CBGA	0	0
CBG	0.240	0.234
CBD	102	99.4
THCV	0.0887	0.0864
CBN	0.196	0.191
d9-THC	0	0
d8-THC	0	0
CBC	0.0942	0.0920
THCA	0	0
d8-THCV	0	0
<b>Total Cannabinoids</b>	<b>102.6</b>	<b>100.0</b>

\*C<sub>n</sub> = C<sub>m</sub> / 102.6 × 100%

## 15 REFERENCES

- 15.1 Shimadzu Cannabis Analyzer for Potency Quick Guide
- 15.2 Shimadzu Potency Testing in Cannabis Extracts Using a High Sensitivity Method with the Cannabis Analyzer for Potency
- 15.3 Maida, V., & Daeninck, P. J. (2016). A user's guide to cannabinoid therapies in oncology. Current oncology (Toronto, Ont.), 23(6), 398-406.
- 15.4 DOC-106, Non-Conformance Report, NCTL.
- 15.5 SOP-120 Cannabinoids Preparation Instructions.
- 15.6 SOP-125, Preparation of Marijuana Flower Samples for Cannabinoid Quantification.
- 15.7 SOP-126, Concentrates Sample Preparation for Cannabinoids Quantification
- 15.8 SOP-127, Sample Preparation of Infused Products by QuEChERS for Cannabinoids Quantification.
- 15.9 SOP-128, Infused Fat-Based Confectionaries Sample Preparation for Cannabinoids Quantification.
- 15.10 SOP-129, Syrup and Honey Sample Preparation for Cannabinoids Quantification.
- 15.11 SOP-130, Infused Beverages Sample Preparation for Cannabinoids Quantification.
- 15.12 SOP-132, Topicals and Tinctures Preparation for Cannabinoids Quantification.
- 15.13 SOP-133, Transdermal Patch Sample Preparation for Cannabinoids Quantification.

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- 15.14 SOP-141 Preparation of Gummy Samples by Methanol Extraction for Cannabinoids Quantification.
- 15.15 SOP-146, Preparation of Chocolate Syrup and Squeeze Gel for Cannabinoids Quantification.
- 15.16 SOP-232, Sample Receiving, NCTL.
- 15.17 SOP-234, Cannabis Waste, NCTL.
- 15.18 SOP-233, Sample Storage, NCTL.
- 15.19 SOP-302, Chemical Hygiene Plan, General Lab Safety Program, NCTL.
- 15.20 LabSolutions: Data Acquisition & Processing Theory Guide. Shimadzu Corp. 2017.

## 16 REVISION HISTORY

Revision Number	Effective Date	Description of Changes
00	07/06/2023	New SOP
01	8/11/2023	M. Wagner, Revised section 6.2 to allow for alternative vendors for cannabinoid standards.
02	10/31/2023	M. Wagner:  Revised Section 9.12 to include the procedure for extrapolation of the calibration curve to 300 µg/mL.  Inserted confirmations procedures to their own Sections 11 – 13 to include further statistical analysis in the determination of confirmations, renumbered subsequent sections.  Made minor grammatical corrections throughout the document.
03	5/20/2024	M. Wagner: Section 15 removed SOP-131 and SOP-134 as references and added SOP-141 and SOP-146. N. Szabo: Section 3.7 corrected spelling for the CBG abbreviation; Removed the UV abbreviation. Section 4.1.1 removed the UV detector reference from the instrumentation used.  Section 4.3.1 changed 2.5 -25 mL dispenser to 1 - 10 mL; Inserted Section 4.3.2 to add 5 – 50 mL dispenser and renumbered subsequent sections.  Added Sections 6.9.1.10 and 6.9.2.8 to include the procedure for adjusting packages in METRC to account for material used for the LCS.  Sections 9.11.1 and 14.3 removed Δ8-THCV from the calculation for total THC.

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Revision Number	Effective Date	Description of Changes
04	9/10/2024	M. Wagner, Eliminated the use of the SSC/SSS from the entire SOP. Changed running the CCV/CCB from 10 to every 20 samples. Updated ICV/CCV formulation to produce a larger amount in sections 6.3 and 6.4. Updated calibration table to exclude a separate calibration for d8-THCV. Now using d9-THCV as a surrogate. Created a formulation for a 100 µg/mL d8-THCV standard in section 6.5 to be run after a calibration and to be used to check and or adjust the correction factor.
05	4/21/2025	<ol style="list-style-type: none"> <li>1. Edited §14.2.2 to calculate results on “as-is” basis rather than on dry-weight basis.</li> <li>2. Edited §14.3 to exclude Δ9-THCV from calculation of Total THC</li> <li>3. General formatting/typographical edits to §14.2, §14.3, and §14.4.</li> <li>4. Removed reference to SOP-107 for determination of moisture content as §15.5</li> <li>5. Updated §9.11.1 to remove Δ9-THCV from Total THC description.</li> </ol>
06	4/23/2025	<ol style="list-style-type: none"> <li>1. M. Wagner, 4/22/2025. Revised section 9.12 to specify that sample results must fall within the dynamic range of calibration curve.</li> <li>2. Revised 4.3.17 to specify calibrated syringes and updated part numbers accordingly. Calibrated syringes have been ordered. This aspect of the SOP will be effective as soon as the calibrated syringes are received.</li> <li>3. Corrected units throughout (e.g. “ul” -&gt; “µL”, “ml” -&gt; “mL”)</li> </ol>

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## 17 APPENDICES

### 17.1 Instrument Method Parameters

Method Editor (Instrument Parameters)

Normal **Advanced** End Time : 10.00 min Method HighSensitivity\_250PPM.lcm

Data Acquisition LC Time Prog. Pump Detector A Column Oven Controller Autosampler AutoPurge

LC Time Program

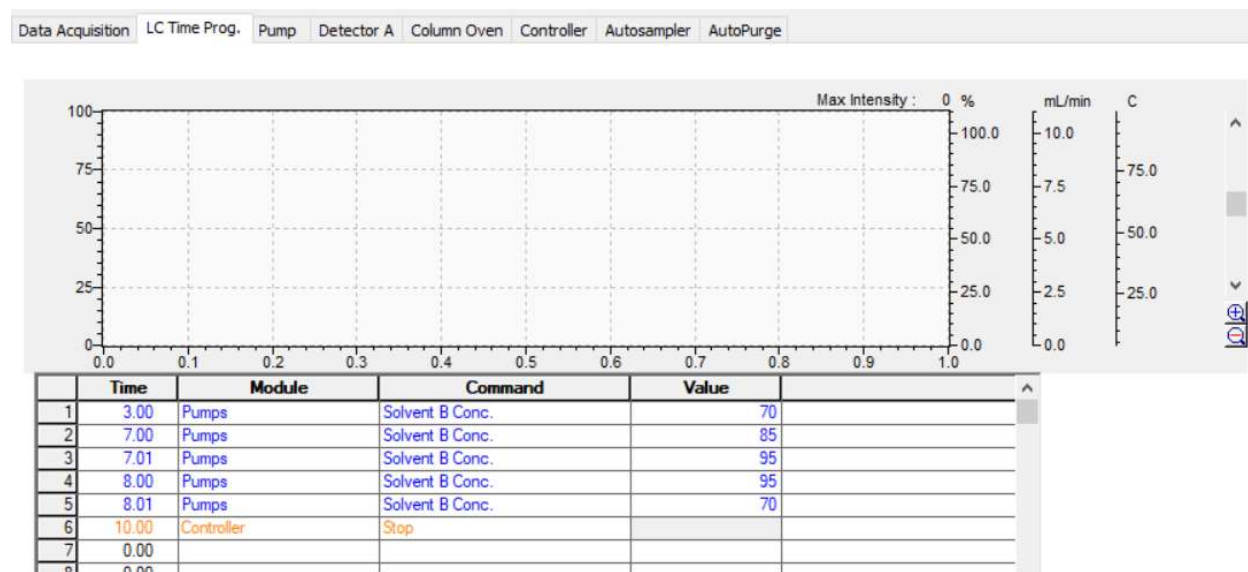
LC Stop Time:  min

☒ Acquisition Time (Detector A)

Sampling: ☐ 5 Hz ☒ 200 msec

Start Time:  min

End Time:  min



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Data Acquisition LC Time Prog. **Pump** Detector A Column Oven Controller Autosampler AutoPurge

Mode: **Low pressure gradient** ▾

Flow:  mL/min

Solvent A Conc.:  % A **Mobile Phase**  ▾

Solvent B Conc.:  % B  ▾

Solvent B Curve:

Solvent C Conc.:  % C  ▾

Solvent C Curve:

Solvent D Conc.:  % D  ▾

Solvent D Curve:

**Configured Pump**

Pump: **LC-2030 Pump**

**Pressure Limits**

Maximum:  psi

Minimum:  psi

**LPGE Control**

LPGE Mode: **Auto** ▾

**Compressibility Setting...**

**Gradient start adjustment**

**None** ▾

Data Acquisition LC Time Prog. **Pump** **Detector A** Column Oven Controller Autosampler AutoPurge

Model: LC-2030 UV Detector

Lamp: **D2** ▾

Polarity: **+** ▾

Response:  sec ▾

☒ Cell Temperature:  C

**Wavelength**

Wavelength Ch1:  nm

**Output**

Intensity Unit: **Volt** ▾

Auxiliary Range1:  AU/V ▾

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Data Acquisition LC Time Prog. Pump Detector A **Column Oven** Controller Autosampler AutoPurge

Model: LC-2030 Oven

☒ Column Oven

Oven Temperature:  C      Temperature Limit(Maximum):  C

Data Acquisition LC Time Prog. Pump Detector A **Column Oven** **Controller** Autosampler AutoPurge

Model: LC-2030 Controller

External output

☐ Event 1

☐ Event 2

☐ Event 3

☐ Event 4

☒ Degassing Unit

Acquisition cycle time optimization

Autosampler pretreatment beginning :  ▾

Pretreatment overlap time :  min

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Data Acquisition LC Time Prog. Pump Detector A Column Oven Controller Autosampler AutoPurge

Model: LC-2030 Autosampler

☒ Autosampler

Injection Settings

Sampling Speed:  uL/sec

Cooler Temperature:  C

Temperature Control

☒ Rack Plate L

☒ Rack Plate R

☐ Air Gap Volume:  uL

Rinse Settings

Rinsing Speed:  uL/sec

Rinsing Volume:  uL

Rinse Mode:  ▾

Rinse Dip Time:  sec

Rinse Solution:

Purge Time (Rinse Port):  min

Data Acquisition LC Time Prog. Pump Detector A Column Oven Controller Autosampler AutoPurge

1 Purge

Purge Order	Mobile Phase Name	Purge Time
1st:	<input type="text" value="Mobile Phase A:0.085%Phosphoric a"/>	<input type="text" value="2"/> min
2nd:	<input type="text" value="Mobile Phase B:0.085%Phosphoric a"/>	<input type="text" value="2"/> min
3rd:	<input type="text" value="Mobile Phase C"/>	<input type="text" value="2"/> min
4th:	<input type="text" value="Mobile Phase D"/>	<input type="text" value="2"/> min
<input checked="" type="checkbox"/> Autosampler Purge		<input type="text" value="10.0"/> min
<input type="checkbox"/> Init. Conc.-Replacement:		<input type="text" value="5"/> min

2 Warm up

Total Flow:  mL/min

Wait Time:  min

3 Activate

☒ Activate system after autopurge