Written by/ Date	Reviewed by/ Date	Approved by/ Date
SAS 03/01/23	CP 03-01-23	03/02/23
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1.0 Purpose

This procedure describes the reversed phase HPLC method for the determination of Cannabidivarin (CBDV), Cannabidiolic Acid (CBDA), Cannabigerol (CBG), Cannabidiol (CBD), Cannabinol (CBN), Δ-9-Tetrahydrocannabinol (Δ-9-THC), Cannabichromene (CBC), and Tetrahydrocannabinolic Acid (THCA) in raw materials and finished products. Additionally, it describes the ID of 8 other cannabinoids

2.0 Scope

This procedure applies to the assay and identification of CBDV, CBDA, CBG, CBD, CBN, Δ-9-THC, CBC and THCA in raw materials and finished products, validated under Protocols MV-LAB-19-044, MV-LAB-19-087 and MV-LAB-19-112. In addition, 8 other cannabinoids have been identified by retention time and referenced to CBD (RRT).

3.0 Responsibility

3.1 It is the responsibility of QC and Analytical Chemists who have verified their ability to execute this procedure to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure, ensure that the procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **HPLC/UV** – High Pressure Liquid Chromatography w/ Ultraviolet Detection

4.2 **QC** – Quality Control

4.3 **CofA** – Certificate of Analysis

4.4 **RRT** – Relative response time

4.5 **IPA** – Isopropyl alcohol

4.6 **HCO_2H** – Formic acid

5.0 References

5.1 MV-LAB-19-044, Protocol, Cannabinoid Determination & ID By HPLC Using UV/Vis Spectroscopy

5.2 MV-LAB-19-087, Protocol, Cannabigerol and Cannabidiolic Acid Determination and Identification using HPLC/UV

5.3 MV-LAB-19-112, Protocol, Cannabidivarin (CBDV), Cannabinol (CBN) and Cannabichromene (CBC) Determination and Identification using HPLC/UV

5.4 USP General Chapter <621> Chromatography

6.0 Reagents, Supplies, Glassware and Equipment

6.1 Reagents

6.1.1 Water, MilliQ

6.1.2 ACN, HPLC Grade

6.1.3 IPA, ACS Grade or Better

6.1.4 Ammonium Formate, ACS Grade or Better

6.1.5 Formic Acid, ACS Grade or Better

6.1.6 CBDV, CBDA, CBG, CBD, CBN, Δ-9-THC, CBC, THCA Reference Standard Solution(s), 1 mg/ml (Restek, Cerilliant or Equivalent)

6.1.7 CBD Bulk Isolate (Extract Labs or Equivalent, Qualified In-House)

6.2 Supplies and Glassware

6.2.1 HPLC vials, 12mm X 32mm with screw cap enclosures w/ septa

6.2.2 Vacuum filtration apparatus with 0.45µ membrane filters

6.2.3 Class "A" volumetric pipets, flasks and graduated cylinders

6.2.4 Glass media bottles

6.2.5 Plastic Luer lock syringes

6.2.6 Nylon syringe filters, 0.45µ

6.2.7 Plastic transfer pipets

6.2.8 Teflon stir bars

6.3 Equipment

6.3.1 Suitable gradient HPLC system consisting of a pump, autosampler, column oven and UV detector with a chromatographic data handling system

6.3.2 Analytical Balance

6.3.3 Stir Plate

7.0 Procedure

7.1 Mobile Phase Preparation

7.1.1 Mobile Phase A (5 mM Ammonium Formate, 0.1% Formic Acid)

7.1.1.1 Combine 1000 mL of water with 1.0 mL of formic acid. Add about 0.3153g of ammonium formate. Stir until dissolved then vacuum filter.

7.1.2 Mobile Phase B (ACN, 0.1% Formic Acid)

7.1.3 Combine 1000 mL of Acetonitrile with 1.0 mL of formic acid. Mix well.

7.1.4 Extraction Solvent = Diluent = IPA

7.2 Standard Prep

7.2.1 CBD – Using a volumetric pipet, transfer 1.0 ml of the (ambient temperature) CBD Reference Standard Solution into a 25 ml volumetric flask. QS to volume with Diluent, mix well, this is the CBD Working Standard (Std A). The nominal concentration of the CBD Working Standard is 40 µg/ml. Prep an additional standard (Std B) as a standard check.

7.2.2 (Minor Component) CBDV, CBDA, CBG, CBN, Δ-9-THC, CBC and THCA – Using a volumetric pipet, transfer 1.0 ml of the (ambient temperature) required Reference Standard Solution into a 50-mL volumetric flask. QS to volume with Diluent, mix well. This is the Minor Component Stock Standard (Stock Std A). Finally, transfer 6.0 mL of the Minor Component Stock Standard into a 50ml volumetric flask. QS to volume with Diluent, mix well. This is the Minor Component Working Standard (Std A). The nominal concentration of the Minor Component Working Standard is 2.4 µg/ml in each cannabinoid. (Alternatively, inject only the THC standard and quantify using the relative response factors provided in Section 7.8 below.) Prep an additional standard (Std B) as a standard check, starting with the stock preparation.

7.2.3 Alternative dilution schemes are permitted.

7.3 Sample Preparation- Assay (Prepare a Single Preparation)

7.3.1 Specific sample testing details are provided in each products profile. If a specific testing details section is not available, then follow preparation procedure as described below.

7.3.2 CBD Stock Sample Solution

7.3.2.1 Determine the size of the volumetric flask to be used for the CBD Stock Sample Solution (V_{stk}):

Sample Strength (% CBD)	V_{stk} (mL)
< 5	25
5-10	50
11-30	100

7.3.2.2 Calculate the sample weight needed for the Stock Sample Solution:

$$SW = \frac{40 \times V_{stk}}{\%CBD}$$

7.3.2.3 Place the empty, volumetric flask on the balance and press Tare. Transfer the amount of sample calculated above into the volumetric flask taking care not to get sample on the sides of the flask. Record the weight of the sample. QS to volume with Extraction Solvent, and mix well. This is the CBD Sample Stock Solution.

7.3.3 CBD Sample Solution

7.3.3.1 Using a glass pipet, transfer 5.0 mL of the Sample Stock Solution into a 50-mL volumetric flask. QS to volume with Diluent, and mix well.

7.3.4 Minor Component Sample Solution

7.3.4.1 The intent of the Minor Component Sample preparation is to ensure that the ratio of the standard concentration to the sample concentration is ~0.1%, which corresponds with the LOQ of the method for both THC and THCA.

7.3.4.2 Weigh ~ 125mg sample into a 50-ml volumetric flask. Dilute to volume with Diluent and mix well.

7.4 HPLC Parameters

7.4.1 Column: Agilent Poroshell 120 SB-C18, 4.6 x 150mm, 2.7µ (Or Equivalent)

7.4.2 Column Temperature: 30°C

7.4.3 Flow rate: 1.5 ml/min

7.4.4 Wavelength: 228 nm (CBDV, CBD, CBN, THC, CBC, THCA, and CBG), 307 nm (CBDA), *285 nm (CBN and CBC) – Optional*

7.4.5 Injection Volume: 5µL

7.4.6 Run Time: 11 min

7.4.7 Recommended 3-D Spectral Range (For Identification): 210nm to 350nm

7.4.8 Mobile Phase: Isocratic 25% A / 75% B

7.5 Recommended Sequence

7.5.1 Make at least 2 injections of the Diluent.

7.5.2 Make five (5) injections of Working Standard A.

7.5.3 Make two (2) injections of Working Standard B.

7.5.4 Make a single injection of each Sample Preparation.

7.5.5 Make a single injection of the Standard Solution after every ten (10) sample injections or at the end of a run.

7.6 System Suitability Requirements

7.6.1 The %RSD of the first five (5) CBD standard A injections is NMT 2.0%.

7.6.2 The calculated recovery of Std A using Std B is 98-102% for CBD (Major component) standards.

7.6.3 The %RSD of all CBD standard injections is NMT 3.0%.

7.6.4 The %RSD of the first five (5) Minor Component standard A injections is NMT 7.0%.

7.6.5 The calculated recovery of Std A using Std B is 93-107% for the Minor Component Standards.

7.6.6 The %RSD of all Minor Component standard injections is NMT 8.0%.

7.6.7 Adjustments allowed in USP <621> are permitted.

7.7 Calculations

7.7.1 $$\mathrm{CBD}\left(\%\frac{w}{w}\right) = \frac{R_u}{R_S} \times C_{Std} \times \frac{V_{Stock}}{SW} \times \frac{mg}{1000\mu g} \times \frac{V_{spl}}{Aq_{spl}} \times 100$$

R_u Sample Peak Area

R_s Mean Standard Peak Area

C_{std} CBD Standard Concentration (µg/mL)

V_{stock} Stock Sample Solution Volume (mL)

SW Sample Weight (mg)

V_{spl} Sample Solution Volume (mL)

Aq_{spl} Aliquot of Stock Sample used to prepare Sample (mL)

7.7.2 $$\mathrm{THC}\left(\%\frac{w}{w}\right) = \left(\frac{R_u}{R_S} \times C_{Std} \times \frac{V_{Stock}}{SW} \times \frac{mg}{1000\mu g} \times \frac{V_{spl}}{Aq_{spl}} \times 100\right) + (0.877 \times THCA)$$

R_u Sample Peak Area

R_s Mean Standard Peak Area

C_{std} THC Standard Concentration (µg/mL)

V_{stock} Stock Sample Solution Volume (mL)

SW Sample Weight (mg)

V_{spl} Sample Solution Volume (mL)

Aq_{spl} Aliquot of Stock Sample used to prepare Sample (mL)

THCA Assay of THCA calculated as in Section 7.7.1 $\left(\%\frac{w}{w}\right)$

7.7.3 $$\text{Minor Component}\left(\%\frac{w}{w}\right) = \frac{R_u}{R_S} \times C_{Std} \times \frac{V_{Stock}}{SW} \times \frac{mg}{1000\mu g} \times \frac{V_{spl}}{Aq_{spl}} \times RRF \times 100$$

R_u Sample Peak Area

R_s Mean Standard Peak Area

C_{std} Standard Concentration (µg/mL)

V_{stock} Stock Sample Solution Volume (mL)

SW Sample Weight (mg)

V_{spl} Sample Solution Volume (mL)

Aq_{spl} Aliquot of Stock Sample used to prepare Sample (mL)

RRF Relative Response Factor (Use THC Peak Area @ 228 nm)

$$\text{Minor Component}\left(\%\frac{w}{w}\right) = \frac{R_u}{R_S} \times C_{Std} \times \frac{V_{Stock}}{SW} \times \frac{mg}{1000\mu g} \times \frac{V_{spl}}{Aq_{spl}} \times RRF \times 100$$

7.8 Relative Response Factors for Minor Component Assays

Phytocannabinoid	Wavelength	Established RRF
THC	228	1.0000
CBDV	228	0.8388
CBDA	307	2.5189
CBG	228	0.8162
CBN	228	0.3948
CBN	285	0.5741
CBC	228	0.4141
CBC	285	1.1690
THCA	228	0.5273

7.9 Relative Retention Times

Phytocannabinoid	Established RRT
CBDVA	0.617
CBDV	0.671
CBDA	0.864
CBGA	0.924
CBG	0.948
CBD	1.000
THCV	1.115
CBN	1.533
d-9-THC	1.929
CBNA	1.981
d-8-THC	1.988
CBL	2.281
CBC	2.440
THCA	2.667
CBCA	3.086
CBLA	3.164

Note: CBCA and CBLA are typically not observed in samples, and elute after the prescribed 11 minute method run time. If desired, the method run time may

be extended to 13 minutes in order to confirm the presence/absence of these species.

7.10 Reporting Results

7.10.1 The expanded uncertainty for CBD is 5.8%.

7.10.2 The expanded uncertainty for THC and THCa is 9.5%.

7.10.3 The expanded uncertainty for all other minor components is 14.8%.

7.10.4 The Coverage factor for all analytes is 2.

7.10.5 Report results along with the expanded uncertainty and coverage factor in the following format (example for CBD):

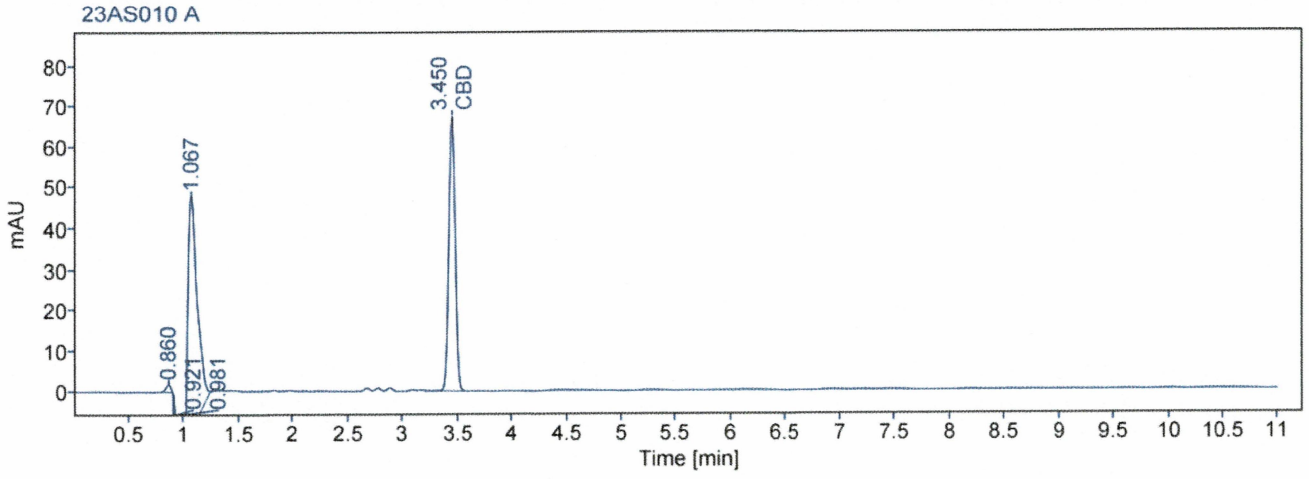
7.10.5.1 108% of Label Claim, U = ± 5.8% k = 2

7.11 Column Wash & Storage

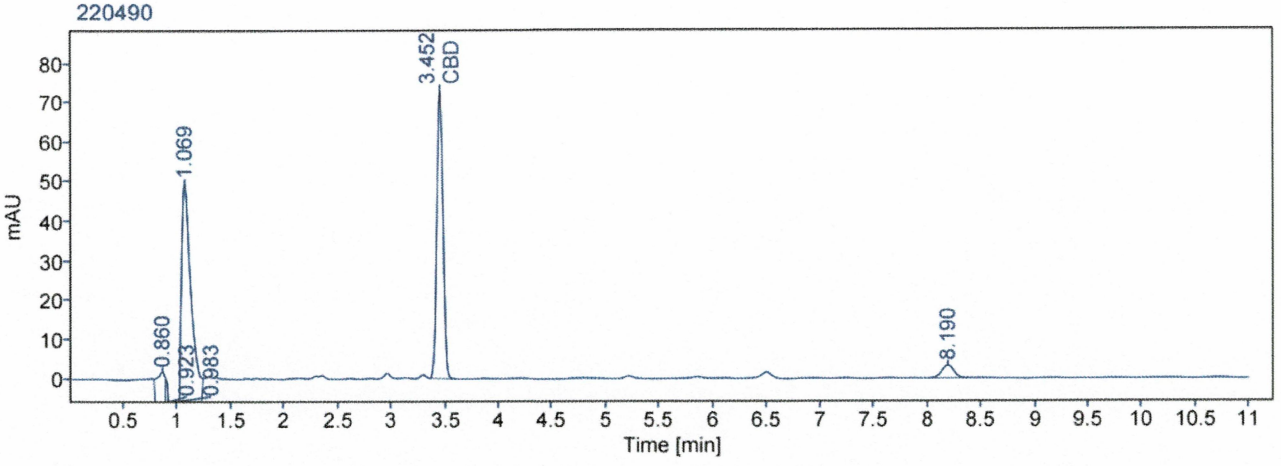
7.11.1 Flush / store the column with / on 75% ACN / 25% Water.

8.0 Chromatograms

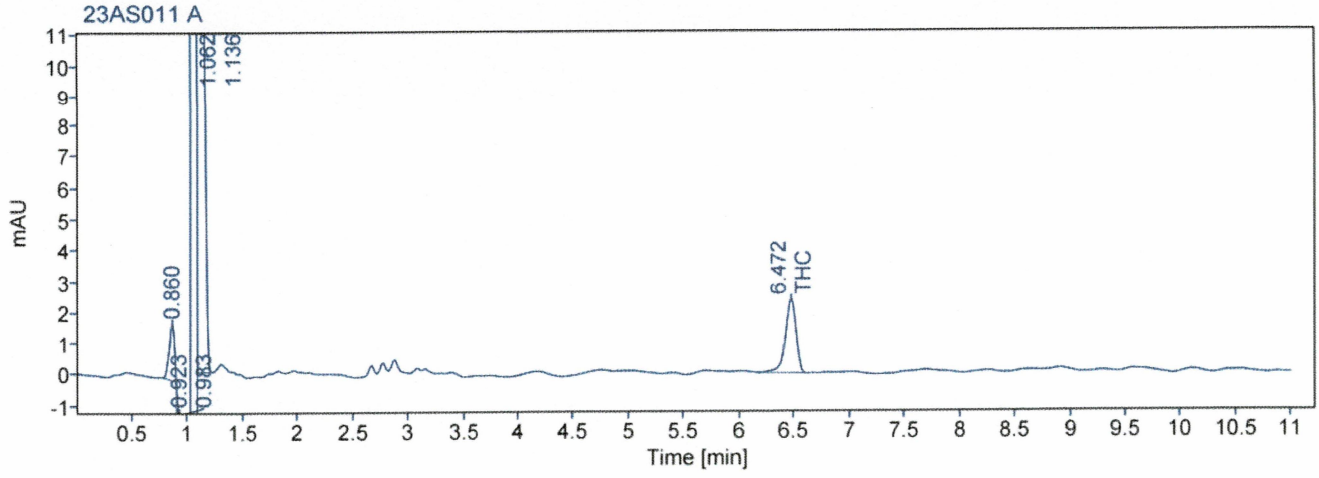
8.1 Typical CBD Working Standard Chromatogram



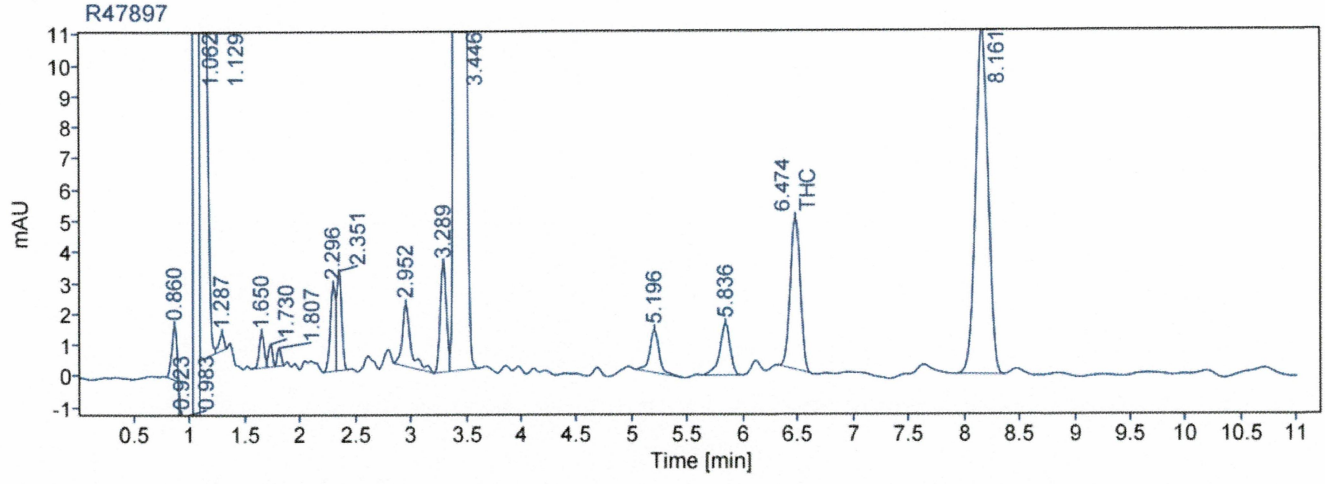
8.2 Typical CBD Sample Chromatogram



8.3 Typical Minor Component Working Standard Chromatogram



8.4 Typical Minor Component Sample Solution Chromatogram



9.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	06/26/19	New	N/A	C. Perry
1	10/26/21	Revised to include ISO 17025 requirements.	CC-21-0396	J. Sassman
2	02/23/23	Add instruction to check the product profile for test details, make it easier to see text in example chromatograms.	CC-23-0092	S. Sassman