This method describes the analysis of samples derived from *Cannabis* species, specifically the assay of the primary compounds of regulatory interest [Tetrahydrocannabinolic Acid (THCA), Tetrahydrocannabinol (THC), Cannabidiolic Acid (CBDA) and Cannabidiol (CBD)] by UPLC coupled with UV or PDA.

Note: This method is adapted from the Cannabis Flos Monograph, produced by the Netherlands Cannabis Bureau.

Test:	*Assay of Cannabinoids* by UPLC		
Column:	Waters Acquity BEH C18, 1.7µm, 2.1 x 150mm, with an Acquity VanGuard BEH C18, 1.7 µm, 2.1 x 5mm guard column		
Mobile phase:	A: 0.1% formic acid in water (0.055 µScm-1), prepared daily B: 0.1% formic acid in acetonitrile		
Gradient table:	**Time (min)**	**A (%)**	**B (%)**
	0.0	30	70
	6.0	30	70
	10.5	0	100
	10.7	0	100
	1.0	30	70
	14	30	70
Wash solvents:	80% acetonitrile (needle wash and purge solvent) 10% acetonitrile (seal wash)		
Flow rate:	0.4 mL/min		
Injection volume:	1 µL		
Column Temp:	30°C		
Sample Temp:	8°C		
Detection:	UV at 228 nm, PDA range 190-400nm with monitoring at 228 nm. Mass Detection may be used if further verification of peak identity is required.		
Sample diluent:	30:70 Mobile phase A:Mobile Phase B		

Resolution Solution

Prepare a solution containing 0.04mg/mL delta-9-THC and 0.005mg/mL delta-8-THC in Diluent. The resolution between the peaks attributed to these compounds in the chromatogram obtained from analysis of this solution must be not less than 1.2.

Standard Preparation (in duplicate)

Prepare the standards in sample diluent for plant material samples.

Prepare the standards in 2-propanol (IPA) for cannabis oil finished product samples.

Weigh accurately an amount of cannabidiol (CBD) and accurately dilute to a volume in the appropriate diluent to achieve a final concentration of 100 µg/mL CBD (this target concentration may need to be adjusted depending on your validated linear range).

Sample Preparation

Plant material:

Perform a loss on drying test on the sample as follows:

Samples should be dried to anhydrous at 40 °C, under vacuum of 1.5 - 2.5kPa (15 -25 mbar), over phosphorus pentoxide. Typically constant weight is achieved within 24 hours. Refer to BP Appendix IX D.

The anhydrous plant material can be homogenised by milling or chopping. Care should be taken to avoid loss of resin through adhesion to surfaces and ensure the sample is not subjected to heat or light.

Immediately following performing the loss on drying, weigh accurately, in triplicate, approximately 1.0 g of anhydrous homogenised sample material into a new plastic 50 mL centrifuge tube. Add a single stainless steel ball (approximately 12 mm diameter), 40mL of ethanol and cap securely. Place on a mechanical shaker at 70% maximum speed for 15 minutes. Centrifuge at 3000rpm for 5 minutes, and transfer the clear upper layer to a 100mL volumetric flask. Repeat this extraction a further two times using 25mL aliquots of ethanol. Make the sample solution to the mark with ethanol.

Filter the solution using a glass fibre / 0.2µm PTFE syringe filter. Dilute the filtrate appropriately with sample diluent to achieve target concentrations within the validated linear range for the target cannabinoids.

Cannabis Oil/ Oral solution:

Density Measurements:

Measure the density of any oral solution and/or cannabis oil sample using a densitometer. Use the density result to calculate the weight equivalent to 1 mL of sample for assay testing.

Cannabis oil capsules:

Prepare a composite of 20 capsules. Measure the average fill weight of the capsules following the procedure in BP Appendix XII C. Use the average fill weight for assay preparation.

Weigh accurately, in triplicate, approximately 1 mL of oral solution/cannabis oil, or the average fill weight for capsules into a 50 mL volumetric flask. Add 40 mL of 2-propanol (IPA) to each volumetric flask and cap securely. Place on a mechanical shaker at 70% maximum speed for 15 minutes. Sonicate for 5 minutes, and make the sample solution to the mark with IPA.

Filter the solution using a glass fibre / 0.2µm PTFE syringe filter. Dilute appropriately with IPA to achieve target concentrations within the validated linear range for the target cannabinoids.

Blank Preparation

Prepare a blank preparation by following the sample preparation steps, omitting the sample.

Calculations:

Plants:

$$\begin{pmatrix}\%\ w/w\ cannabinoid\\(anhydrous\ basis)\end{pmatrix} = \frac{Area_{cannabinoid}}{Area_{standard}} \times \frac{wt_{standard}}{wt_{sample}} \times DF \times P(\%) \times RRF$$

$$\begin{pmatrix}\%\ w/w\ cannabinoid\\(as\ is)\end{pmatrix} = \begin{pmatrix}\%\ w/w\ cannabinoid\\(anhydrous\ basis)\end{pmatrix} \times LOD\ factor$$

$$\begin{pmatrix}Total\ CBD\\(\%\ w/w)\end{pmatrix} = \%\ w/w\ CBDA \times 0.877 + \%\ w/w\ CBD$$

$$\begin{pmatrix}Total\ THC\\(\%\ w/w)\end{pmatrix} = \%\ w/w\ THCA \times 0.877 + \%\ w/w\ THC$$

Cannabis Oil Finished Products:

$$\begin{pmatrix}amount\ cannabinoid\\(mg/mL)\end{pmatrix} = \frac{Area_{cannabinoid}}{Area_{standard}} \times \frac{wt_{standard}}{wt_{sample}} \times DF \times P \times RRF \times Den$$

$$\begin{pmatrix}Total\ CBD\\(mg/mL)\end{pmatrix} = amount\ CBDA \times 0.877 + amount\ CBD$$

$$\begin{pmatrix}Total\ THC\\(mg/mL)\end{pmatrix} = amount\ THCA \times 0.877 + amount\ THC$$

% Stated content:

$$\begin{pmatrix}\%\ stated\ content\\(CBD)\end{pmatrix} = \frac{total\ CBD\ amount\ or\ \%w/w}{stated\ content\ CBD} \times 100\ \%$$

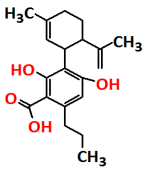
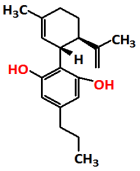
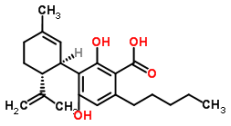
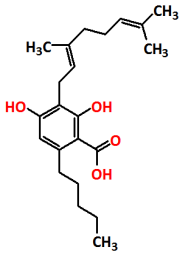
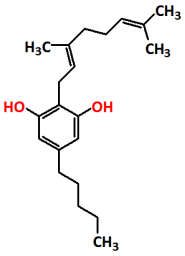
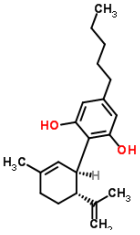
$$\begin{pmatrix}\%\ stated\ content\\(THC)\end{pmatrix} = \frac{total\ THC\ amount\ or\ \%w/w}{stated\ content\ THC} \times 100\ \%$$

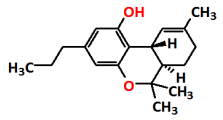
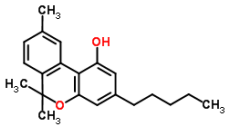
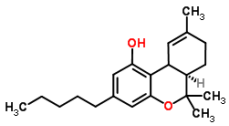
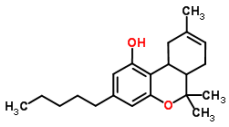
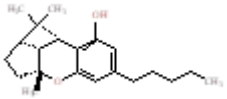
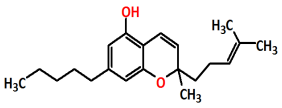
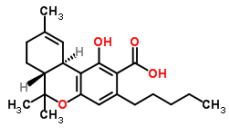
Where:

$Area_{Cannabinoid}$	is the peak area response of the individual cannabinoid in the sample solution
$Area_{Standard}$	is the average peak area response of CBD in the standard solution
$Wt_{Standard}$	is the weight of CBD reference material, in mg
Wt_{Sample}	is the weight of sample, in mg
DF	is the combined standard and sample dilution factor
P(%)	is the 'as is' potency in percentage of the CBD reference material
P	is the 'as is' potency in fraction form of the CBD reference material
RRF	is the relative response factor of the individual cannabinoid to CBD – Refer to Table 1.
LOD factor	is the loss on drying expressed in fraction form ((100 – loss on drying (%))/100)
Den	is the density measurement in mg/mL

Similar calculations for other cannabinoids can be performed, using the values listed in Table 1.

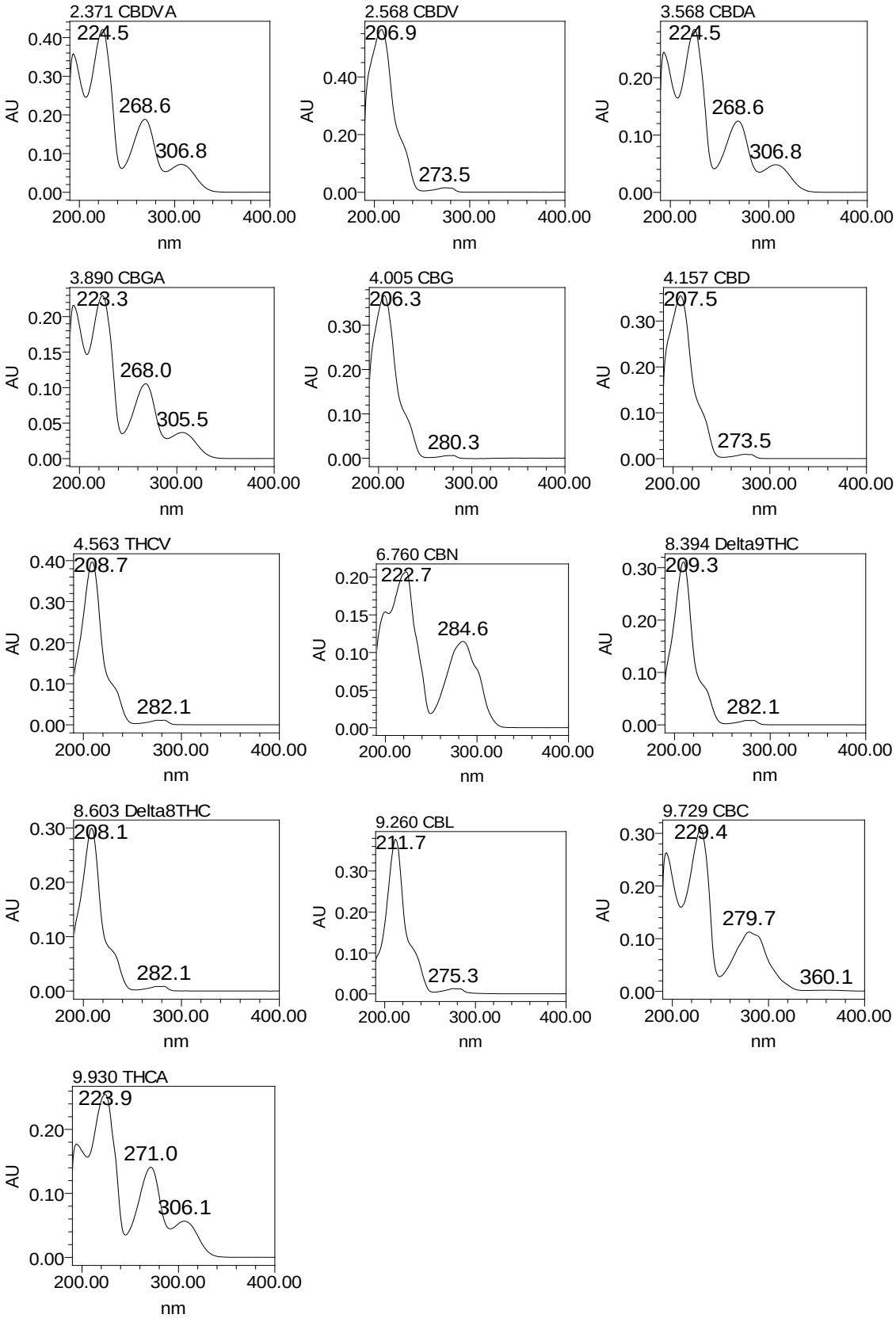
Table 1: RRTs and RRFs for UPLC

Component	Structure	Relative Retention Time	Relative Response Factor (RRF)	MS mode & parent ion mass
Cannabidivarinic Acid (CBDVA)		0.57	0.496	-ve mode 329.2
Cannabidivarin (CBDV)		0.62	0.923	+ve mode 287.2
Cannabidiolic Acid (CBDA)		0.86	0.538	-ve mode 357.3
Cannabigerolic Acid (CBGA)		0.93	0.534	-ve mode 359.3
Cannabigerol (CBG)		0.97	1.069	+ve mode 317.2
Cannabidiol (CBD)		1.00	1.000	+ve mode 315.2

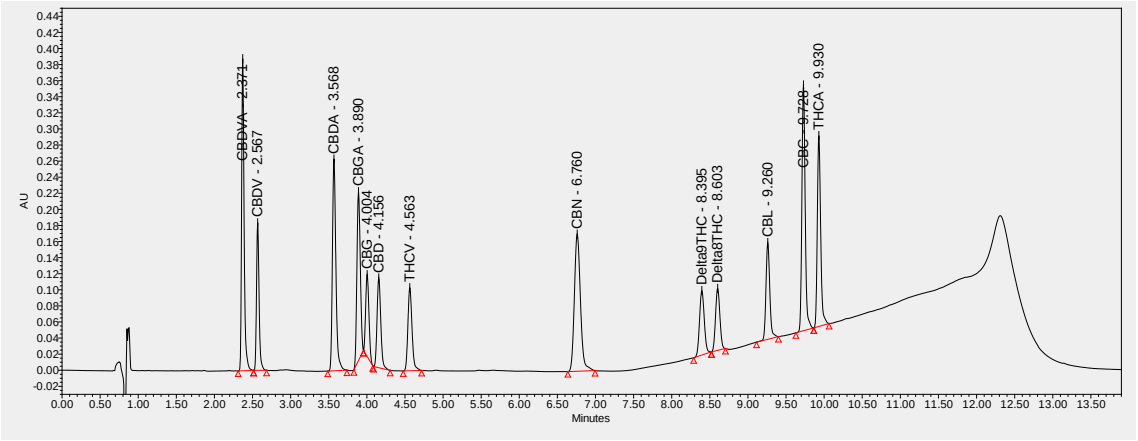
Component	Structure	Relative Retention Time	Relative Response Factor (RRF)	MS mode & parent ion mass
Tetrahydrocannabivarin (THCV)		1.10	1.064	+ve mode 287.2
Cannabinol (CBN)		1.63	0.421	+ve mode 311.2
Δ^9-Tetrahydrocannabinol (THC)		2.02	1.124	+ve mode 315.2
Δ^8-Tetrahydrocannabinol (Δ^8-THC)		2.07		+ve mode 315.2
(+/-) Cannabicyclol (CBL)		2.23	1.039	+ve mode 315.2
Cannabichromeme (CBC)		2.35	0.453	+ve mode 315.2
Tetrahydrocannabinolic Acid (THCA)		2.39	0.588	-ve mode 357.3

This table provides information on the standards currently validated for linearity. If further investigation is required for a peak in the assay both positive and negative ionisation modes should be considered. The intensity of [M+H]+ ions for acid-type cannabinoids in positive ion mode tends to be low as in-source fragmentation can occur. [M-H]- ions have been observed in negative ion mode for the acid-type cannabinoids.

UV Spectra:


2.371 CBDVA
224.5
268.6
306.8
2.568 CBDV
206.9
273.5
3.568 CBDA
224.5
268.6
306.8
3.890 CBGA
223.3
268.0
305.5
4.005 CBG
206.3
280.3
4.157 CBD
207.5
273.5
4.563 THCV
208.7
282.1
6.760 CBN
222.7
284.6
8.394 Delta9THC
209.3
282.1
8.603 Delta8THC
208.1
282.1
9.260 CBL
211.7
275.3
9.729 CBC
229.4
279.7
360.1
9.930 THCA
223.9
271.0
306.1
AU
nm

Example Chromatogram



Mixed Cannabinoids Standard Solution ~ 70 µg/mL, chromatogram extracted at 228 nm.