

OLEOLITES (PHYTOCANNABINOIDS FOR THERAPEUTIC USE) by LC/MS

Code LC88810





This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD).

The declaration of conformity (CE) is available upon request.



EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com

N° 003	Phytocannabinoids for therapeutic use by LC/MS	07/2024
--------	--	---------

INTRODUCTION

The pharmacist may be asked to prepare galenic preparations of therapeutic cannabis in compliance with standard rules of preparation. This implies that the active substance will be present in the dose and form of medication indicated by the prescriber.

The substance can be inhaled through the use of a specific vaporizer or it can undergo an oral administration in the case of a decoction.

Currently, there are no studies that shed light on the side effects or on the acute toxicity of herbal preparations which are defined as 'oil' or 'oil solution' of cannabis. Such preparations contain unspecified cannabis extracts in oil or other solvents.

According to the current legislation the quality of the product must be guaranteed.

Therefore, the titration of the active ingredient/s must be carried out for each galenic preparation by using sensitive methodologies such as liquid or gas chromatography coupled with mass spectrometry.

The intended use of the method shown here is to titrate galenic oily preparations which contain cannabinoids such as:

- THC: TETRAHYDROCANNABINOL
- CBD: CANNABIDIOL
- CBN: CANNABINOL
- CBG: CANNABIGEROLO
- THCA: TETRAHYDROCANNABINOLIC ACID
- CBDA: CANNABIDIOLIC ACID
- THCV: TETRAHYDROCANNABIVARINE

MATRIX EFFECT:	Tetrahydrocannabinol (THC) Cannabidiol (CBD) Cannabigerol (CBG) Cannabinol (CBN) Tetrahydrocannabivarine (THCV) Cannabidiolic Acid (CBDA) Tetrahydrocannabinolic Acid (THCA)	125,0% 117,1% 115,7% 83,0% 122,7% 116,7% 114,4%									
SENSITIVITY (LLOD):	Tetrahydrocannabinol (THC) Cannabidiol (CBD) Cannabigerol (CBG) Cannabinol (CBN) Tetrahydrocannabivarine (THCV) Cannabidiolic Acid (CBDA) Tetrahydrocannabinolic Acid (THCA)	0,03 mg/ml 0,03 mg/ml 0,01 mg/ml 0,1 mg/ml 0,03 mg/ml 0,01 mg/ml 0,01 mg/ml									
MINIMUM CONCENTRATION ANALYZABLE (LLOQ):	Tetrahydrocannabinol (THC) Cannabidiol (CBD) Cannabigerol (CBG) Cannabinol (CBN) Tetrahydrocannabivarine (THCV) Cannabidiolic Acid (CBDA) Tetrahydrocannabinolic Acid (THCA)	0,1 mg/ml 0,1 mg/ml 0,05 mg/ml 0,5 mg/ml 0,1 mg/ml 0,05 mg/ml 0,05 mg/ml									
LINEARITY:	Tetrahydrocannabinol (THC) Cannabidiol (CBD) Cannabigerol (CBG) Cannabinol (CBN) Tetrahydrocannabivarine (THCV) Cannabidiolic Acid (CBDA) Tetrahydrocannabinolic Acid (THCA)	0,1 – 200 mg/ml 0,1 – 200 mg/ml 0,05 – 200 mg/ml 0,5 – 200 mg/ml 0,1 – 200 mg/ml 0,05 – 200 mg/ml 0,05 – 200 mg/ml									
Accuracy intra serie (relative error %) Tetrahydrocannabinol:	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>4,02%</td><td>2,68%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	4,02%	2,68%				
Ci	Cs										
2,5 mg/ml	10 mg/ml										
4,02%	2,68%										
Accuracy inter serie (relative error %) Tetrahydrocannabinol:	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>6,26%</td><td>5,08%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	6,26%	5,08%				
Ci	Cs										
2,5 mg/ml	10 mg/ml										
6,26%	5,08%										
Reproducibility intra serie (coefficient of variation %) Tetrahydrocannabinol:	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,1 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>7,70%</td><td>2,73%</td><td>2,93%</td></tr></table>	C LLOQ	Cm	CUP	0,1 mg/ml	5 mg/ml	20 mg/ml	7,70%	2,73%	2,93%	
C LLOQ	Cm	CUP									
0,1 mg/ml	5 mg/ml	20 mg/ml									
7,70%	2,73%	2,93%									
Reproducibility inter serie (coefficient of variation %) Tetrahydrocannabinol:	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,1 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>13,25%</td><td>6,84%</td><td>9,16%</td></tr></table>	C LLOQ	Cm	CUP	0,1 mg/ml	5 mg/ml	20 mg/ml	13,25%	6,84%	9,16%	
C LLOQ	Cm	CUP									
0,1 mg/ml	5 mg/ml	20 mg/ml									
13,25%	6,84%	9,16%									
Coefficient of Correlation R2 + Dev Std:	0,9966 ± 0,0022 Tetrahydrocannabinol										
Accuracy intra serie (relative error %) Cannabidiol:	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>5,22%</td><td>3,29%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	5,22%	3,29%				
Ci	Cs										
2,5 mg/ml	10 mg/ml										
5,22%	3,29%										

<u>Accuracy intra serie (relative error %)</u> <u>Cannabidiol:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>6,46%</td><td>5,94%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	6,46%	5,94%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
6,46%	5,94%									
<u>Reproducibility intra serie (coefficient of variation %)</u> Cannabidiol:	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,1 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>11,52%</td><td>4,47%</td><td>4,92%</td></tr></table>	C LLOQ	Cm	CUP	0,1 mg/ml	5 mg/ml	20 mg/ml	11,52%	4,47%	4,92%
C LLOQ	Cm	CUP								
0,1 mg/ml	5 mg/ml	20 mg/ml								
11,52%	4,47%	4,92%								
<u>Reproducibility inter serie (coefficient of variation %)</u> Cannabidiol:	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,1 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>14,82%</td><td>8,96%</td><td>8,88%</td></tr></table>	C LLOQ	Cm	CUP	0,1 mg/ml	5 mg/ml	20 mg/ml	14,82%	8,96%	8,88%
C LLOQ	Cm	CUP								
0,1 mg/ml	5 mg/ml	20 mg/ml								
14,82%	8,96%	8,88%								
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9971 ± 0,0014 Cannabidiol									
<u>Accuracy intra serie (relative error %)</u> <u>Cannabigerol:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>2,48%</td><td>2,83%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	2,48%	2,83%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
2,48%	2,83%									
<u>Accuracy inter serie (relative error %)</u> <u>Cannabigerol:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>5,59%</td><td>6,62%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	5,59%	6,62%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
5,59%	6,62%									
<u>Reproducibility intra serie (coefficient of variation %)</u> Cannabigerol:	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,05 ng/ml</td><td>5 ng/ml</td><td>20 ng/ml</td></tr><tr><td>4,28%</td><td>4,31%</td><td>5,48%</td></tr></table>	C LLOQ	Cm	CUP	0,05 ng/ml	5 ng/ml	20 ng/ml	4,28%	4,31%	5,48%
C LLOQ	Cm	CUP								
0,05 ng/ml	5 ng/ml	20 ng/ml								
4,28%	4,31%	5,48%								
<u>Reproducibility inter serie (coefficient of variation %)</u> Cannabigerol:	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,05 ng/ml</td><td>5 ng/ml</td><td>20 ng/ml</td></tr><tr><td>10,76%</td><td>9,87%</td><td>8,46%</td></tr></table>	C LLOQ	Cm	CUP	0,05 ng/ml	5 ng/ml	20 ng/ml	10,76%	9,87%	8,46%
C LLOQ	Cm	CUP								
0,05 ng/ml	5 ng/ml	20 ng/ml								
10,76%	9,87%	8,46%								
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9947 ± 0,0036 Cannabigerol									
<u>Accuracy intra serie (relative error %)</u> <u>Cannabinol:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>5,97%</td><td>4,69%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	5,97%	4,69%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
5,97%	4,69%									
<u>Accuracy inter serie (relative error %)</u> <u>Cannabinol:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>6,67%</td><td>6,53%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	6,67%	6,53%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
6,67%	6,53%									
<u>Reproducibility intra serie (coefficient of variation %)</u> Cannabinol:	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,5 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>6,97%</td><td>6,59%</td><td>4,29%</td></tr></table>	C LLOQ	Cm	CUP	0,5 mg/ml	5 mg/ml	20 mg/ml	6,97%	6,59%	4,29%
C LLOQ	Cm	CUP								
0,5 mg/ml	5 mg/ml	20 mg/ml								
6,97%	6,59%	4,29%								
<u>Reproducibility inter serie (coefficient of variation %)</u> TCannabinol:	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,5 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>8,54%</td><td>6,56%</td><td>9,55%</td></tr></table>	C LLOQ	Cm	CUP	0,5 mg/ml	5 mg/ml	20 mg/ml	8,54%	6,56%	9,55%
C LLOQ	Cm	CUP								
0,5 mg/ml	5 mg/ml	20 mg/ml								
8,54%	6,56%	9,55%								
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9950 ± 0,0043 Cannabinol									
<u>Accuracy intra serie (relative error %)</u> <u>Tetrahydrocannabivarin:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>6,19%</td><td>2,98%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	6,19%	2,98%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
6,19%	2,98%									

<u>Accuracy inter serie (relative error %)</u> <u>Tetrahydrocannabivarin:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>7,59%</td><td>5,69%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	7,59%	5,69%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
7,59%	5,69%									
<u>Reproducibility intra serie (coefficient of variation %)</u> <u>Tetrahydrocannabivarin:</u>	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,1 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>7,89%</td><td>1,03%</td><td>2,36%</td></tr></table>	C LLOQ	Cm	CUP	0,1 mg/ml	5 mg/ml	20 mg/ml	7,89%	1,03%	2,36%
C LLOQ	Cm	CUP								
0,1 mg/ml	5 mg/ml	20 mg/ml								
7,89%	1,03%	2,36%								
<u>Reproducibility inter serie (coefficient of variation %)</u> <u>Tetrahydrocannabivarin:</u>	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,1 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>12,58%</td><td>8,71%</td><td>6,06%</td></tr></table>	C LLOQ	Cm	CUP	0,1 mg/ml	5 mg/ml	20 mg/ml	12,58%	8,71%	6,06%
C LLOQ	Cm	CUP								
0,1 mg/ml	5 mg/ml	20 mg/ml								
12,58%	8,71%	6,06%								
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9964 ± 0,0024 Tetrahydrocannabivarin									
<u>Accuracy intra serie (relative error %)</u> <u>Cannabidiolic Acid:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>3,03%</td><td>1,37%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	3,03%	1,37%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
3,03%	1,37%									
<u>Accuracy inter serie (relative error %)</u> <u>Cannabidiolic Acid:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>7,33%</td><td>5,57%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	7,33%	5,57%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
7,33%	5,57%									
<u>Reproducibility intra serie (coefficient of variation %)</u> <u>Cannabidiolic Acid:</u>	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,05 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>6,11%</td><td>3,42%</td><td>4,22%</td></tr></table>	C LLOQ	Cm	CUP	0,05 mg/ml	5 mg/ml	20 mg/ml	6,11%	3,42%	4,22%
C LLOQ	Cm	CUP								
0,05 mg/ml	5 mg/ml	20 mg/ml								
6,11%	3,42%	4,22%								
<u>Reproducibility inter serie (coefficient of variation %)</u> <u>Cannabidiolic Acid:</u>	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,05 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>12,83%</td><td>6,06%</td><td>5,30%</td></tr></table>	C LLOQ	Cm	CUP	0,05 mg/ml	5 mg/ml	20 mg/ml	12,83%	6,06%	5,30%
C LLOQ	Cm	CUP								
0,05 mg/ml	5 mg/ml	20 mg/ml								
12,83%	6,06%	5,30%								
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9949 ± 0,0012 Cannabidiolic Acid									
<u>Accuracy intra serie (relative error %)</u> <u>Tetrahydrocannabinolic Acid:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>3,64%</td><td>5,01%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	3,64%	5,01%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
3,64%	5,01%									
<u>Accuracy inter serie (relative error %)</u> <u>Tetrahydrocannabinolic Acid:</u>	<table><tr><td>Ci</td><td>Cs</td></tr><tr><td>2,5 mg/ml</td><td>10 mg/ml</td></tr><tr><td>6,82%</td><td>6,54%</td></tr></table>	Ci	Cs	2,5 mg/ml	10 mg/ml	6,82%	6,54%			
Ci	Cs									
2,5 mg/ml	10 mg/ml									
6,82%	6,54%									
<u>Reproducibility intra serie (coefficient of variation %)</u> <u>Tetrahydrocannabinolic Acid:</u>	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,05 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>7,16%</td><td>6,41%</td><td>4,91%</td></tr></table>	C LLOQ	Cm	CUP	0,05 mg/ml	5 mg/ml	20 mg/ml	7,16%	6,41%	4,91%
C LLOQ	Cm	CUP								
0,05 mg/ml	5 mg/ml	20 mg/ml								
7,16%	6,41%	4,91%								
<u>Reproducibility inter serie (coefficient of variation %)</u> <u>Tetrahydrocannabinolic Acid:</u>	<table><tr><td>C LLOQ</td><td>Cm</td><td>CUP</td></tr><tr><td>0,05 mg/ml</td><td>5 mg/ml</td><td>20 mg/ml</td></tr><tr><td>12,89%</td><td>6,15%</td><td>6,38%</td></tr></table>	C LLOQ	Cm	CUP	0,05 mg/ml	5 mg/ml	20 mg/ml	12,89%	6,15%	6,38%
C LLOQ	Cm	CUP								
0,05 mg/ml	5 mg/ml	20 mg/ml								
12,89%	6,15%	6,38%								
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9942 ± 0,0018 Tetrahydrocannabinolic Acid									

COMPONENTS OF THE KIT (100 TESTS)

Reagent A – Extraction Solution	2 x 500 ml
Reagent B – Diluting Solution	1 x 100 ml
Reagent C – Stabilization Solution + deuterated IS	1 x 100 ml <u>Store at -20 °C</u>
Calibrator – Level 0	1 x 300 µl Code LC88816 (Packed separately – see data sheets)
Calibrator – Level 1	1 x 300 µl Code LC88816 (Packed separately – see data sheets)
Calibrator – Level 2	1 x 300 µl Code LC88816 (Packed separately – see data sheets)
Calibrator – Level 3	1 x 300 µl Code LC88816 (Packed separately – see data sheets)
Calibrator – Level 4	1 x 300 µl Code LC88816 (Packed separately – see data sheets)
Calibrator – Level 5	1 x 300 µl Code LC88816 (Packed separately – see data sheets)
Reagent M1 – Mobile Phase M1	1 x 500 ml
Reagent M2 – Mobile Phase M2	2 x 500 ml

All reagents are ready to use and stable for 3 years at room temperature except Reagent C which should be stored at -20 °C and so it is stable for 3 months.

MINIMUM INSTRUMENTAL EQUIPMENT REQUIRED

LC-MS/MS Triple Quadrupole

ESI Interface

OPTIONAL EQUIPMENT

Autosampler

Operational Computer

METHOD OF COLLECTING AND STORING THE SAMPLE***N.A.******These are oils of galenic production for therapeutic purposes.*****ACCESSORIES AND CONSUMABLES**

CODE	DESCRIPTION	PACKAGING
LC88816	Calibrator for Phytocannabinoids	6 x 2 x 300 µl
LC88819	Control for Phytocannabinoids – Levels 1 and 2	2 x 1 x 500 µl
TF25402052130	Hypersil GOLD PFP (50 x 2,1mm x 1,9µm) Analytical Column	1 Pc
L-30-210	Eppendorf da 1,5 ml con tappo	1 x 1000 Pcs
S29057U	Standard Glass Vials of 2 ml	1 x 100 Pcs
Z1636/26	Pyrex Tubes of 10 ml	1 x 40 Pcs

ANALYTICAL PROCEDURE

PROCEDURE FOR OIL SAMPLES

STEP 1

Dispense in pyrex tube:

- 100 µl of oil
- 9900 µl of Reagent A – Extraction Solution

Vortex for 30 sec.

STEP 2

Dispense in eppendorf tubes:

- 10 µl of supernatant
- 990 µl of Reagent B – Diluting Solution

Vortex for 10 sec.

STEP 3

Dispense in vial:

- 50 µl of sample of Step 2
- 950 µl of Reagent C – Stabilization Solution + Internal Standards

Vortex for 10 sec.

INJECTION

- Inject 2 - 10 µl according to instrumental sensitivity

PROCEDURE FOR CALIBRATION CURVE

Dispense in vial:

- 50 µl of Calibrators (C0-C5)/Controls
- 950 µl of Reagent C – Stabilization Solution + Internal Standards

N.B.: at this step, the sample is stable 24 hours at -20 °C

INJECTION

- Inject 2 - 10 µl according to instrumental sensitivity

COLUMN CONDITIONING

Install the new Hypersil Gold PFP analytical column (50 x 2.1 mm, 1.9 µm), thermostated at 40°C. Disconnect the detector and pass 30 ml of a solution of H₂O : Methanol (20 : 80 v/v) at a flow of 300 µl / minute. Condition further by flushing a solution of H₂O : Mobile Phase M2 (95 : 5 v/v) at a flow of 400 µl for 15 minutes. Do not recycle washing liquids. Two injections of 50% HPLC / 50% Acetonitrile grade water before proceeding with the analytical series.

Phase recirculation analysis is NOT possible.

If the Laboratory T Amb is 20 °C it is recommended to store the Mobile Phase at 2-8 °C between analytical sessions.

COLUMN CLEANING

Flush a solution of H₂O : Methanol (20 : 80 v/v) for 30 minutes by discharging it. The column should be stored in a Mobile Phase M1 solution: Mobile Phase M2 (50 : 50 v/v).

INJECTION NEEDLE WASHING

Wash the injection needle with Methanol.

GRADIENT SET

Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0.0	75	25	400
1.0	75	25	400
8.0	0	100	400
11.0	0	100	400
11.1	75	25	400
12.0	75	25	400
15.0	75	25	400

PARAMETERS SET ON TRIPLE QUADRUPOLE SCIEX 4500 ESI POSITIVE

Curtain Gas	25
Collision Gas	10
Ion Spray Voltage	1500
Temperature (°C)	450
Ion Source Gas 1	55
Ion Source Gas 2	55

PARAMETERS SET ON TRIPLE QUADRUPOLE SCIEX 4500 ESI NEGATIVE

Curtain Gas	25
Collision Gas	10
Ion Spray Voltage	-1500
Temperature (°C)	450
Ion Source Gas 1	55
Ion Source Gas 2	55

FRAGMENTATIONS

MOLECULE	IS	Q1	Q3	RT	POLARITY
THC	THC D3	315.3	193.1	7.5	+
CBD	CBD D3	315.3	193.1	6.9	+
CBG	CBN D3	317.1	193.1	6.9	+
CBN	CBN D3	311.3	293.1	7.3	+
THCV	THC D3	287.1	165.3	6.9	+
CBDA	CBD D3	357.2	339.1	7.0	-
THCA	THC D3	357.2	339.1	7.9	-
THC D3	-	318.3	123.1	7.5	+
CBD D3	-	318.3	123.1	6.9	+
CBN D3	-	314.3	223.1	7.3	+

PHYTOCANNABINOIDS by LC/MS

(Reference Chromatograms)

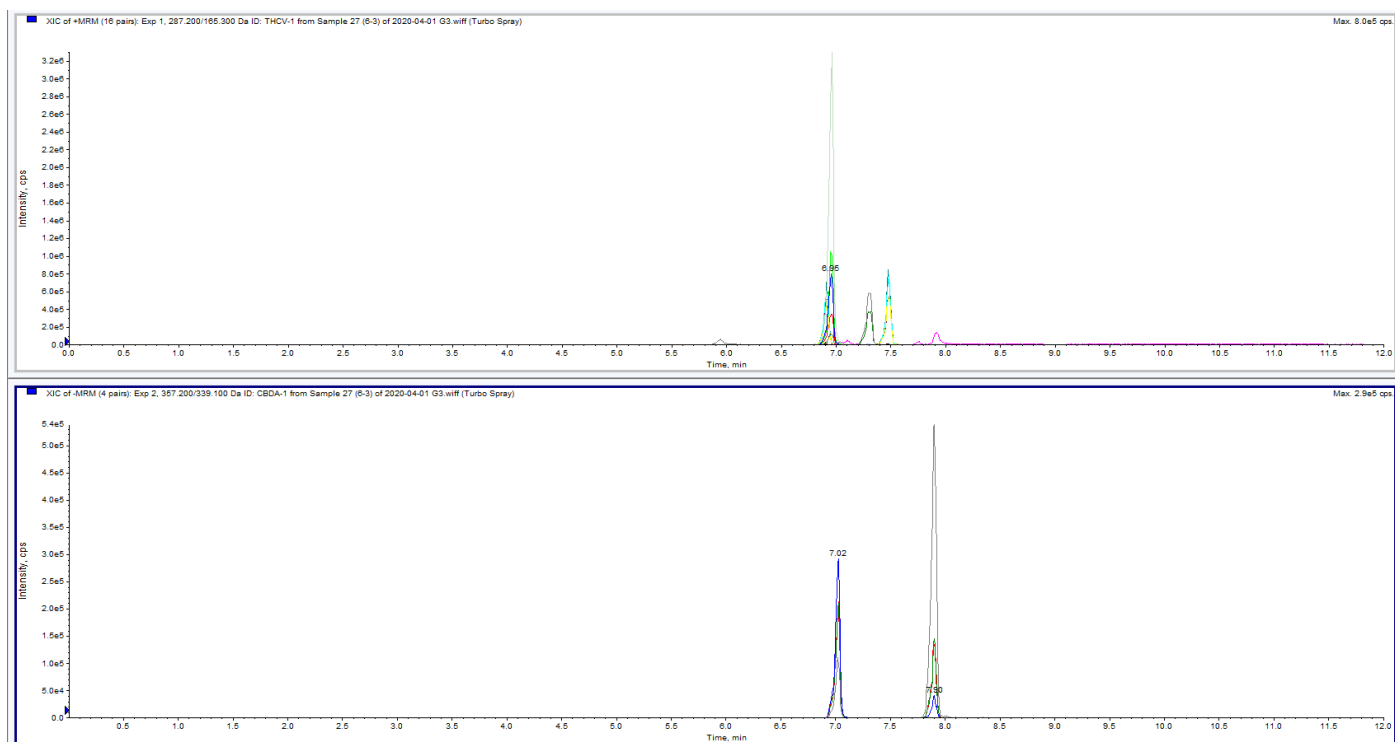


Fig 1 Calibrator Level 5 for Phytocannabinoids, at the top the molecules that ionize in positive mode (THC, THCV, CBD, CBG, CBN), at the bottom those that ionize in negative mode (THCA and CBDA)

PHYTOCANNABINOIDS by LC/MS

(Reference Chromatograms)

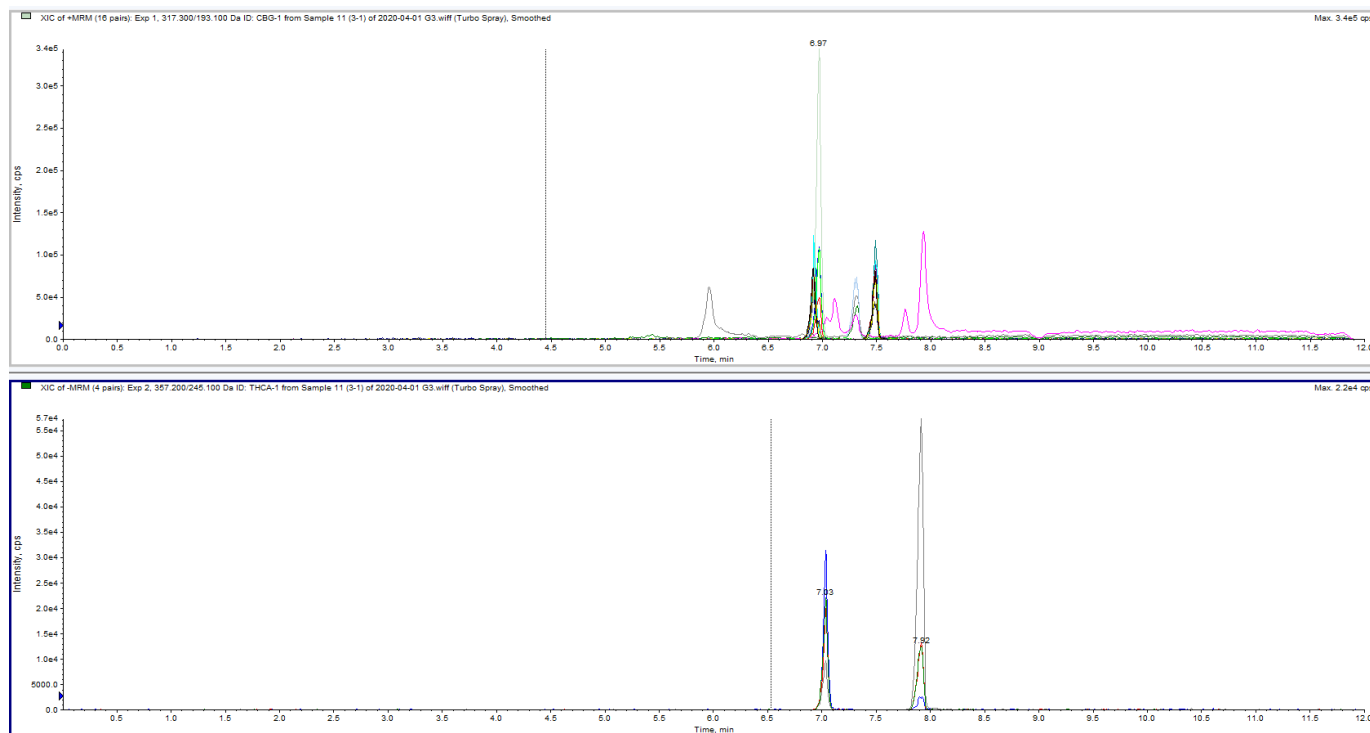


Fig 2 Control level 2 for Phytocannabinoids, at the top the molecules that ionize in positive mode (THC, THCV, CBD, CBG, CBN), at the bottom those that ionize in negative mode (THCA and CBDA)

PHYTOCANNABINOIDS by LC/MS

(Reference Chromatograms)

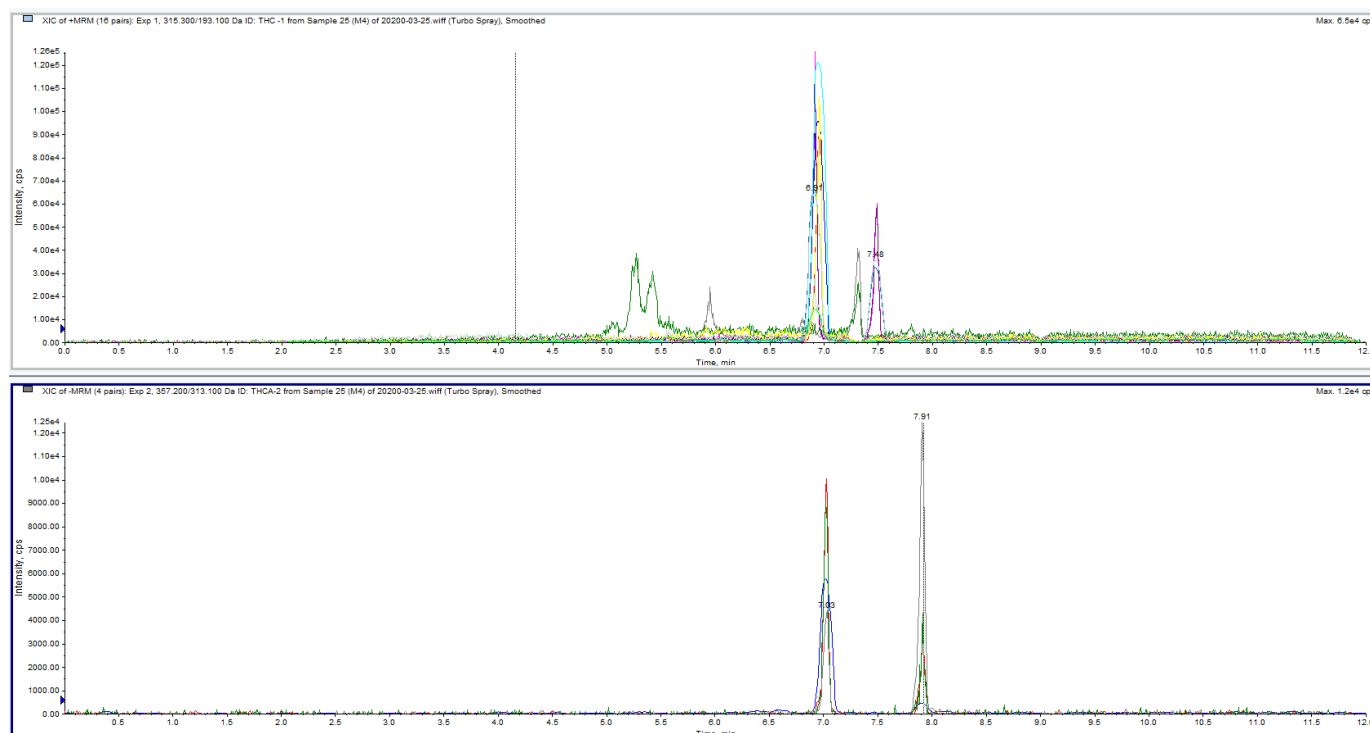


Fig 3 Positive Sample for THC, CBD, CBDA, THCA

References

- B. Murnion, Medicinal cannabis, Aust. Prescr. 38 (6) (2015) 212–215, <http://dx.doi.org/10.18773/austprescr.2015.072>.
- L.M. Borgelt, K.L. Franson, A.M. Nussbaum, G.S. Wang, The pharmacologic and clinical effects of medical cannabis Pharmacotherapy 33 (2) (2013) 195–209, <http://dx.doi.org/10.1002/phar.1187>.
- B. Jensen, J. Chen, T. Furnish, M. Wallace, Medical Marijuana and Chronic Pain: a Review of Basic Science and Clinical Evidence, Curr. Pain Headache Rep. 19(10) (2015) 1–9, <http://dx.doi.org/10.1007/s11916-015-0524-x>, article 50.
- G.M. Merone, A. Tartaglia, M. Locatelli, C. D'Ovidio, E. Rosato, U. de Grazia, F. Santavenere, S. Rossi, F. Savini, Analytical Chemistry in the 21st century: challenges, solutions, and future perspectives in complex matrices quantitative analyses in biological/clinical field, Analytica 1 (1) (2020) 44–59, <http://dx.doi.org/10.3390/analytica1010006>.
- M.K. Mohamed, J. Takyi-Williams, B. Baudot, A. Grobler, Development and validation of a LC-HRMS method for the quantification of cannabinoids and their metabolites in human plasma, Eur. J. Pharm. Sci. 159 (2021) 1–8, <http://dx.doi.org/10.1016/j.ejps.2021.105705>, article 105705.