

Test no: 202

Cannabinoids *tested* by *liquid chromatography* In vitro testing for fungi High Performance Thin Layer Cromatography for element analisys SC Petrochem Technologies SRL member of CANNADERM ROMANIA

Navodari, Strada Prelungirea Recoltei 13 Jud Constanta, Romania

BCR, Sucursala Navodari, IBAN: RO47RNCB0119119143090001

Extract Producer: Ferma Domnita Maria (under entity II Petrescu MV Cezar)- ORGANIC CERTIFICATE HOLDER- RO Ro31303888/ F51/107/2013 Dor Marunt Romania Batch No: 21/A/19-H6 +004(0)242643754

This test report contains test result obtained from Qualitative analisys performed by the University of Technology Prague (Anexa 1)- document number ML667/19.

Product Analyzed: 6 bottles of Cannabidiol OIL -CBD oil (Full Plant Extract) as written on the product batch form (high pressure reactor extraction- stripping column). Batch size: 1000l Packaging: 100 ml bottles Content: Cannabis extract – Full plant extract- Decarboxylated Samples received: -03-05-2019 Form of Botanical: liquid product; Specific Density: 0.96 Sample: 3.0; 4.0; 6.0; 7.0; 8.0, 9.0 Analyst: Mihaela Anghelescu Test Methods used for determination of compounds: HPLC-FLD; HRMS; LC-MS

Conclusion: All samples indicated similar results. No fungi infestation from in vitro test.

II Petrescu MV Cezar Ro31303888 F51/107/2013

F51/107/2013 Tel: 0040 (0)732 55 97 88 Cannabis plants origin: Romania

Producator



Quantitative evaluation- method LC-MS

Cannabinoids	Result
CBD (cannabidiol)-anxyolitic/antypsyhotic/analgesic/anti-inflamatory/ antispasmotic/antioxydant	9260 mg/kg (0.9260%)
CBDA (cannabidiolic acid) -antibiotic	94 mg/kg (0.0094%)
Δ9-THC (delta 9 tetrahydrocannabinol)-euphoriant/analgesic/ anti-inflamatory/ antiemetic/antioxydant	400 mg/kg (0.04%)
Δ8-THC (delta 8 tetrahydrocannabinol)- similar to THC- less potent	60 mg/kg (0.006%)
Δ9-THCA-A (delta 9 tetrahydrocannabinolic acid-A)- immuno-modulating effect not modulated by CB1&2 receptors	30 mg/kg (0.003%)
CBN (cannabinol)- Sedative/antibiotic/ antyconvulsant/ anty-inflammatory	33 mg/kg (0.0033%)
CBG (cannabigerol) -analgesic/antibiotic/anti-fungal/anti-inflamatory	101 mg/kg (0.01%)
CBGA (cannabigerolic acid) -antibiotic	4.8 mg/kg
CBDV (cannabidivarine)- neurochemical pathway for previously-observed anti-epileptic and anti-convulsive action	120 mg/kg (0.012%)
CBC (canabichromene)- analgesic/antibiotic/anti-fungal/anti-inflamatory	230 mg/kg (0.023%)
THCV (tetrahydrocannabivarine)- analgesic / euphoriant	68 mg/kg ( 0.0068%)
CBDVA (cannabidivarinic acid) – anti-inflammatory	8 mg/kg
Vit E	143 mg/kg (0.01435)



# Appendix no. 1\* to the test certificate nr 202 (\*extract from VSCHT Prague- test certificat ML:667/19) Records documenting sample analysis using metabolomic fingerprinting U-HPLC-HRMS/MS

## **Testing strategy**

For the purpose of comparison of samples in terms of content of phenolic compounds, oxidized triacyglyceols and tocopherols a strategy focused on analysis of small molecules was selected for samples. Metabolomic fingerprinting realized by ultra-high performance chromatography coupled to high resolution tandem mass spectrometry (U-HPLC-HRMS/MS) was performed and subsequently, database containing 263 compounds was used for targeted screening.

### **Testing conditions**

Samples were i) extracted by 80% methanol, ii) diluted by ethanol. Subsequently, reversed-phase column was used for the separation of compounds and HRMS type quadrupole/time of flight (TripleTOF 6600, SCIEX) was used for detection and PeakView 2.0 software enabled data evaluation (Instrumentation C).

#### **Test results**

Database containing 39 phenolic compounds, 216 oxidized triacylglycerols and 8 tocopherols/tocotrienols was used for targeted screening of samples. In all the samples 17 phenolic compounds, 16 oxidized triacylglycerols and 3 tocopherols were identified. All compounds were identified based on accurate *m/z* value of their molecular ion, isotopic profiles and characteristic fragments (MS/MS spectra) and are summarized in **table I-III**. All the results are also illustrated in **figures 1-3** for better visualization

Table I: Compounds detected in sample	Molecular formula	Monitored adduct	Theoretical <i>m/z</i> value	Measured <i>m/z</i> value	Mass error (ppm)	Intensity
Phenolic compounds ESI+ 4,5-dihydroxy-2,3,6- trimethoxy-9,10- dihydrophenanthrene	C17H18O5	[M+H]+	303.1227	303.12255	-0.5	366259





4-hydroxy-2,3,6,7- tetramethoxy-9,10- dihydrophenanthrene	C18H20O5	[M+H]+	317.13835	317.13844	0.3	9590
6-prenylapigenin	C20H18O5	[M+H]+	339.1227	339.12259	-0.3	5204
apigenin	C15H10O5	[M+H]+	271.0601	271.05793	-8	1405
cannabispiran /	C15H18O3	[M+H]+	247.13287	247.12997	-4.8	449725
isocannabispiran						
cannabispirenone-	C15H16O3	[M+H]+	245.11722	245.11471	-4.3	36780
A/cannabispirenone-						
B/iso-						
cannabispirone/3,4'-						
dihydroxy-5-methoxy bibenzyl						
cannabispirol/alpha-	C15H20O3	[M+H]+	249.14852	249.1455	-4.1	263678
cannabispiranol/beta-	013112003	[].	213.11032	213.1133	1.1	203010
cannabispiranol						
cannabistilbene-I	C20H24O3			[M+H]+	313.17982	ND
cannflavin A / cannflavin	C26H28O6	[M+H]+	437.19587	437.20111	5	158846
С						
cannflavin B	C21H20O6	[M+H]+	369.13327	369.13503	4.8	104530
canniprene	C21H26O4	[M+H]+	343.19039	343.19098	1.7	293940
cannithrene-2	C16H16O4	[M+H]+	273.11214	273.11171	-1.6	82762
chrysoeriol	C16H12O6	[M+H]+	301.07066	301.06968	-3.3	2224
denbinobin	C16H12O5	[M+H]+	285.07575	285.07843	4.4	2769
(iso)cannabispiradienone	C15H14O3	[M+H]+	243.10157	243.09858	-4.9	240653
/ cannithrene-1	C101110NO4	[14.11].	214 12000	214 12045	0.7	60007
Ntrans-feruloyltyramine	C18H19NO4	[M+H]+	314.13868	314.13845	-0.7	68087
phloroglucinol Phenolic compounds ESI-	C6H6O3			[M+H]+	127.03897	ND
Filehouc compounds ESI-						





4,5-dihydroxy-2,3,6- trimethoxy-9,10- dihydrophenanthrene	C17H18O5	[M-H]-	301.10815	301.10831	0.5	144207
4-hydroxy-2,3,6,7- tetramethoxy-9,10- dihydrophenanthrene	C18H20O5	[M-H]-	315.1238	315.12393	0.4	1971
6-prenylapigenin	C20H18O5	[M-H]-	337.10815	337.10787	-0.8	23743
apigenin	C15H10O5	[M-H]-	269.04555	269.04533	-0.8	8655
cannabispiran / isocannabispiran	C15H18O3	[M-H]-	245.11832	245.11844	0.5	23831
cannabispirenone- A/cannabispirenone- B/iso-cannabispirone/3,4- dihydroxy-5-methoxy bibenzyl	C15H16O3	[M-H]-	243.10267	243.10277	0.4	26469
cannabispirol/alpha- cannabispiranol/beta- cannabispiranol	C15H20O3	[M-H]-	247.13397	247.13381	-0.6	6497
cannabistilbene-I	C20H24O3	[M-H]-	311.16527	311.16563	1.2	7168
cannflavin A / cannflavin C	C26H28O6	[M-H]-	435.18131	435.18151	0.5	200137
cannflavin B	C21H20O6	[M-H]-	367.11871	367.11902	0.8	127034
canniprene	C21H26O4	[M-H]-	341.17583	341.17571	-0.4	53491
cannithrene-2	C16H16O4	[M-H]-	271.09758	271.09783	0.9	62964
chrysoeriol	C16H12O6	[M-H]-	299.05611	299.05584	-0.9	7729
denbinobin	C16H12O5	[M-H]-	283.0612	283.06079	-1.4	5019
(iso)cannabispiradienone / cannithrene-1	C15H14O3	[M-H]-	241.08702	241.08722	0.8	39390
Ntrans-feruloyltyramine	C18H19NO4	[M-H]-	312.12413	312.12391	-0.7	19071





phloroglucinol	C6H6O3					ND
Oxidized triacylglycerols	ESI+					
C 50:3;1	C53H96O7	[M+NH4]+	862.74943	862.74796	-1.7	52180
C 50:2;1	C53H98O7	[M+NH4]+	864.76508	864.76292	-2.5	9958
C 52:4;1	C55H98O7	[M+NH4]+	888.76508	888.76483	-0.3	377661
C 52:3;1	C55H100O7	[M+NH4]+	890.78073	890.77953	-1.4	372111
C 54:6;1	C57H98O7	[M+NH4]+	912.76508	912.76427	-0.9	3683388
C 54:5;1	C57H100O7	[M+NH4]+	914.78073	914.78025	-0.5	1795817
C 54:4;1	C57H102O7	[M+NH4]+	916.79638	916.79596	-0.5	1484974
C 54:3;1	C57H104O7	[M+NH4]+	918.81203	918.80322	-6.6	249238
C 56:4;1	C59H106O7	[M+NH4]+	944.82768	944.82333	-4.6	26191
C 56:3;1	C59H108O7	[M+NH4]+	946.84333	946.84058	-2.9	11749
C 52:4;2	C55H98O8	[M+NH4]+	904.76	904.75896	-1.1	178643
C 52:3;2	C55H100O8	[M+NH4]+	906.77565	906.77368	-2.2	96112
C 54:6;2	C57H98O8	[M+NH4]+	928.76	928.75959	-0.4	651479
C 54:5;2	C57H100O8	[M+NH4]+	930.77565	930.77577	0.1	788436
C 54:4;2	C57H102O8	[M+NH4]+	932.7913	932.79052	-0.8	409074
C 54:3;2	C57H104O8	[M+NH4]+	934.80695	934.80445	-2.7	154051
Analyte	Molecular	Monitored	Theoretical <i>m/z</i>	Measured <i>m/z</i>	Mass error (ppm)	Intensity
	formula	adduct	value	value		
Tocopherols ESI+						
alpha - tocopherol	C29H50O2	[M+H]+	431.38836	431.38807	-0.7	567152
beta/gama - tocopherol	C28H48O2	[M+H]+	417.37271	417.37172	-2.4	43190
alpha -	C29H50O3	[M+H]+	447.38327	447.38018	-4.9	15801
tocopherolquinone						

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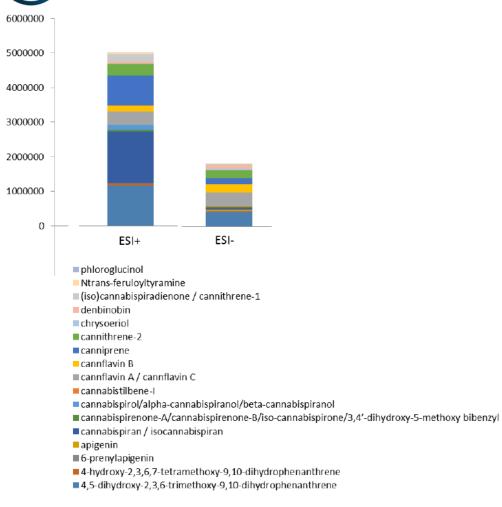
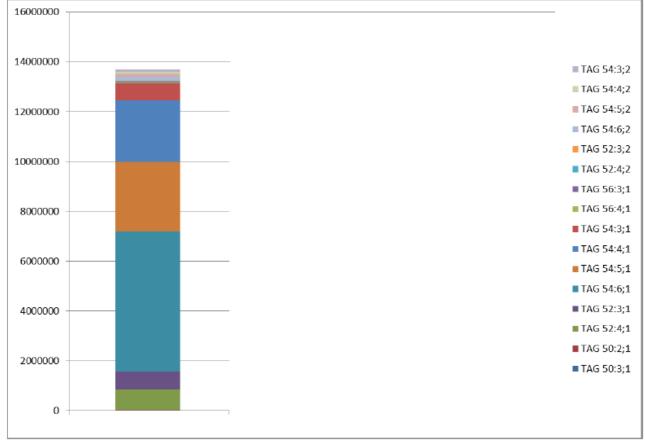


Figure 1: Intensities of phenolic compounds in positive and negative ionization mode

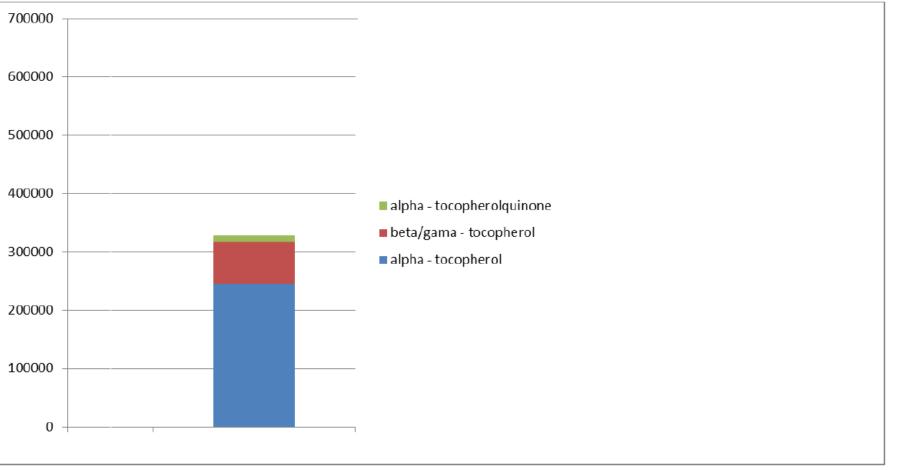






**Figure 2:** Intensities of oxidized triacylglycerols (TAG x:y;z = triacylglycerol; x = number of carbons in bound fatty acid; y = number of double bonds in bound fatty acid; z=number of oxidation) in positive ionization mode.





Forma Domnita Maria produse naturale bio

Figure 3: Intensities of tocopherols in positive ionization mode



#### Interpretation of tests:

All the samples showed very similar metabolomic fingerprints and regarding targeted compounds there were no differences in identified compounds. According to the results, the samples have been fully decarboxylated, and they contain the complete spectrum of cannabinoinds/ phenols/ therpenes/ flavonoids with low content of Vit E.

The ration CBD/THC in this product is 19:1.

The quantitative analisys of Phenolic compounts could not be performed due to lack of calibration charts.

THC/ THCA level has been confirmed by a 3rd party (Romanian Blaj Narcotic Laboratory) and the returned value is 0.06%.