Method Development for the Separation of Major Cannabinoids and Terpenes using a six Column Selectivity Screening Approach

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1. Introduction

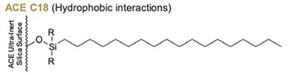
- Cannabis samples are complex and contain several target compound classes that are of interest from analytical and regulatory perspectives.
- Cannabinoids and terpenes are major components and are therefore of primary importance.
- Regulatory requirements for testing are varied and may require determination of several key cannabinoids, for example[1]:
  - Δ^9-Tetrahydrocannabinol (Δ^9-THC)
  - Cannabidiol (CBD)
  - Δ^9-Tetrahydrocannabinolic acid A (THCA-A)
  - Cannabidiolic acid (CBDA)
  - Cannabigerol (CBG)
  - Cannabinol (CBN)
- Terpenes are another target class of interest due to potential synergistic effects with cannabinoids and potential use for fingerprinting cultivars.
- This poster summarises work performed to develop LC-MS compatible methods for the analysis of an extended list of 10 cannabinoids of interest and for fingerprinting terpene content.

[1]State of California, AB 266 Medical marijuana, Article 9, Section 19344 (2015-2016)

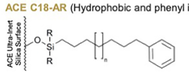
2. Experimental

- Samples were screened on six reversed-phase columns, including five ACE novel chemistries, to identify the most suitable stationary phase chemistry.
- The ACE novel chemistries have been engineered to each offer unique selectivity and provide a comprehensive method development tool.

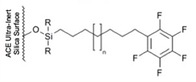
ACE C18 (Hydrophobic interactions)



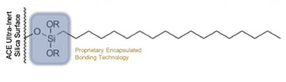
ACE C18-AR (Hydrophobic and phenyl interactions)



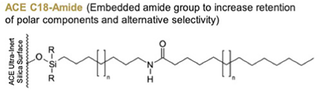
ACE C18-PFP (Hydrophobic and PFP interactions)



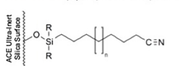
ACE SuperC18 (Highly retentive with extended pH stability)



ACE C18-Amide (Embedded amide group to increase retention of polar components and alternative selectivity)

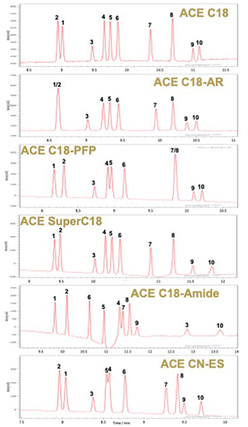


ACE CN-ES (Hydrophobic and polar interactions)



3. Cannabinoids – Six-Column Screen

- Sample was screened on the six ACE phases using a 5-95% gradient.
- LC/MS compatible mobile phase.
- The six ACE phases provide alternative selectivity – ideal for method development.
- The **ACE SuperC18** was found to be the most promising phase.



Conditions

Column Format: 100 x 3.0 mm, 2 µm
Mobile Phase: A: 0.1% formic acid in H_2O
B: 0.1% formic acid in MeCN
Gradient:

Time	0	10	13	13.5
%B	5	95	95	5

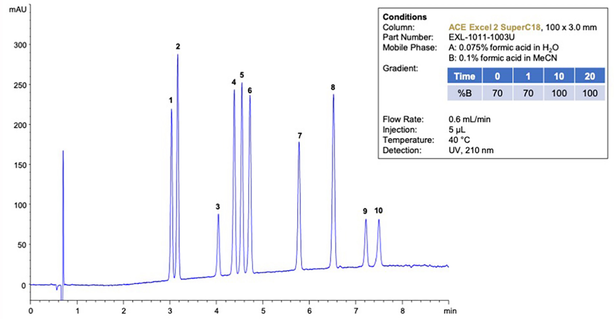
Flow Rate: 0.6 mL/min
Injection: 5 µL
Temperature: 40 °C
Detection: UV, 210 nm

Sample

1. (-)-11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), 2. Cannabidivarin (CBDV), 3. Cannabidiolic acid (CBDA), 4. Cannabigerol (CBG), 5.Cannabidiol (CBD), 6. Tetrahydrocannabivarin (THCV), 7. Cannabinol (CBN), 8. (-)-*trans*-Δ^9-Tetrahydrocannabinol (THC), 9. Cannabichromene (CBC), 10. Δ^9-Tetrahydrocannabinolic acid A (THCA-A)

4. Cannabinoids - Optimised Separation

- The gradient conditions were optimised to reduce analyte retention and maintain resolution of the analytes.
- An initial isocratic hold was required to maintain separation of peaks 1&2.
- Full separation achieved in <8 minutes.



Conditions

Column: ACE Excel 2 SuperC18, 100 x 3.0 mm
Part Number: EXL-1011-1003U
Mobile Phase: A: 0.075% formic acid in H_2O
B: 0.1% formic acid in MeCN
Gradient:

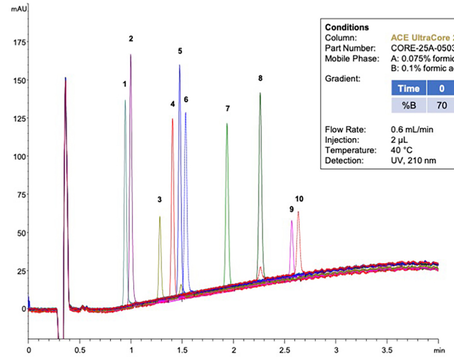
Time	0	1	10	20
%B	70	70	100	100

Flow Rate: 0.6 mL/min
Injection: 5 µL
Temperature: 40 °C
Detection: UV, 210 nm

1. (-)-11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), 2. Cannabidivarin (CBDV), 3. Cannabidiolic acid (CBDA), 4. Cannabigerol (CBG), 5. Cannabidiol (CBD), 6. Tetrahydrocannabivarin (THCV), 7. Cannabinol (CBN), 8. (-)-*trans*-Δ^9-Tetrahydrocannabinol (THC), 9. Cannabichromene (CBC), 10. Δ^9-Tetrahydrocannabinolic acid A (THCA-A)

5. Cannabinoids - Rapid Analysis

- The method was translated to an **ACE UltraCore 2.5 SuperC18** solid core column using the ACE LC Translator Tool (Download free at www.ace-hplc.com).



Conditions

Column: ACE UltraCore 2.5 SuperC18, 50 x 3.0 mm
Part Number: CORE-25A-0503U
Mobile Phase: A: 0.075% formic acid in H_2O
B: 0.1% formic acid in MeCN
Gradient:

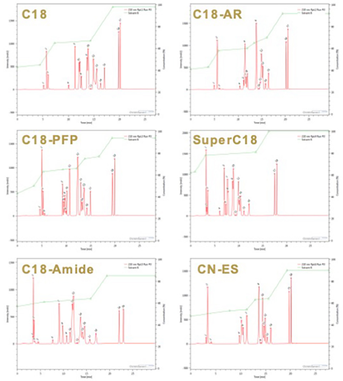
Time	0	0.25	4.18	5.05
%B	70	70	100	100

Flow Rate: 0.6 mL/min
Injection: 2 µL
Temperature: 40 °C
Detection: UV, 210 nm

1. (-)-11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), 2. Cannabidivarin (CBDV), 3. Cannabidiolic acid (CBDA), 4. Cannabigerol (CBG), 5. Cannabidiol (CBD), 6. Tetrahydrocannabivarin (THCV), 7. Cannabinol (CBN), 8. (-)-*trans*-Δ^9-Tetrahydrocannabinol (THC), 9. Cannabichromene (CBC), 10. Δ^9-Tetrahydrocannabinolic acid A (THCA-A)

6. Fingerprinting Terpenes – Six-Column Screening

- A set of 21 terpenes was screened using automated ChromSword Auto 5 method development software to identify optimal gradient profile.
- The six phases give different selectivity for this complex sample – ideal for method development.
- The **ACE C18-PFP** was selected based on retention, peaks separated and overall selectivity.

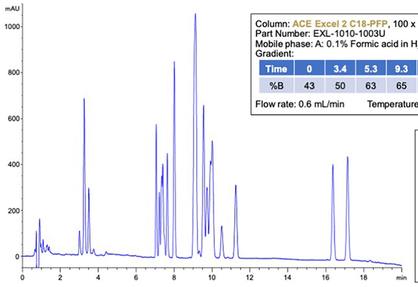


Conditions

Column Format: 100 x 3.0 mm, 2 µm
Mobile Phase: A: 0.1% H_3PO_4 in H_2O
B: 0.1% H_3PO_4 in MeCN
Gradient: Various
Flow Rate: 0.6 mL/min
Injection: 5 µL
Temperature: 40 °C
Detection: UV, 210 nm

7. Terpenes – Optimised Method

- The **ACE C18-PFP** provided reasonable separation for this complex mix.
- Method suitable for fingerprinting terpene profile of cannabis samples.
- Method moved to LC-MS compatible conditions to allow identification and quantitation of individual components as required.
- Future work:
  - Further optimise the separation
  - Develop a unified LC-MS method for cannabinoids and terpenes.



Column: ACE Excel 2 C18-PFP, 100 x 3.0 mm
Part Number: EXL-1010-1003U
Mobile phase: A: 0.1% Formic acid in H_2O B: 0.1% Formic acid in MeCN
Gradient:

Time	0	3.4	5.3	9.3	12.1	13.8	16.7	19.4	28.2
%B	43	50	63	65	66	75	77	94	94

Flow rate: 0.6 mL/min Temperature: 30 °C

Analytes

α-Pinene	Camphene
(-)-β-Pinene	β–Mycene
δ-3-Carene	α-Terpinene
D-Limonene	Ocimene
γ-Terpinene	Terpinolene
Linalool	(-)-Isopulegol
Geraniol	β-Caryophyllene
α-Humulene	Nerolidol
(-)-Guaiol	(-)-α-Bisabolol
p-Isopropyltoluene (p-Cymene)	
1,8-Cineole (Eucalyptol)	
(-)-Caryophyllene oxide	

8. Conclusions

- Full separation of 10 cannabinoids was successfully achieved.
- Method development using six-column screening rapidly identified the **ACE SuperC18** as the optimum stationary phase for the separation.
- Optimisation of the gradient resulted in full separation of the cannabinoids in less than 8 minutes using LC-MS compatible conditions.
- The final separation was translated to a 50 x 3.0 mm format **ACE UltraCore 2.5 SuperC18** column to further reduce the analysis time to < 3 minutes.
- An LC-MS compatible method for terpenes was identified on the **ACE Excel C18-PFP** using a column screening approach.
- Work is ongoing to develop a unified method for the determination of both cannabinoids and terpenes in a single run.