Development of a Method for the Separation of Major Cannabinoids Using Six Column Selectivity Screening

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1. Introduction

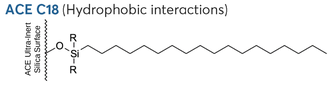
- Cannabinoids are a major compound class and are therefore of primary importance.
- Cannabis samples are complex and contain several target compound classes that are of interest from analytical and regulatory perspectives.
- Regulatory requirements for testing are varied and may require determination of several key components, 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)
- This poster summarises work performed to develop an LC-MS compatible method for the analysis of an extended list of 10 cannabinoids of interest.

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

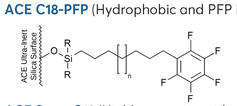
2. Experimental

- The sample was 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 provide unique selectivity and provide a comprehensive method development tool.

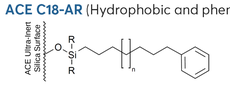
ACE C18 (Hydrophobic interactions)



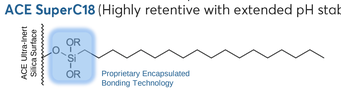
ACE C18-PFP (Hydrophobic and PFP interactions)



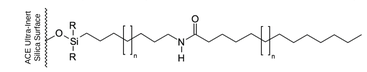
ACE C18-AR (Hydrophobic and phenyl interactions)



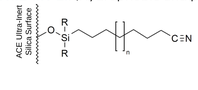
ACE SuperC18 (Highly retentive with extended pH stability)



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



ACE CN-ES (Hydrophobic and polar interactions)



3. Six Column Screening

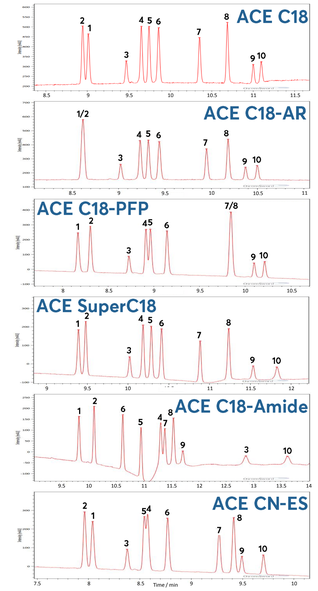
- Sample was screened on the six ACE phases using a 5-95% gradient.
- LC/MS compatible mobile phase.
- The six ACE phases provide alternative selectivity – ideal for method development.
- The ACE SuperC18 was found to be the most promising phase.

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 (mins)	%B
0.0	5
10.0	95
13.0	95
13.5	5

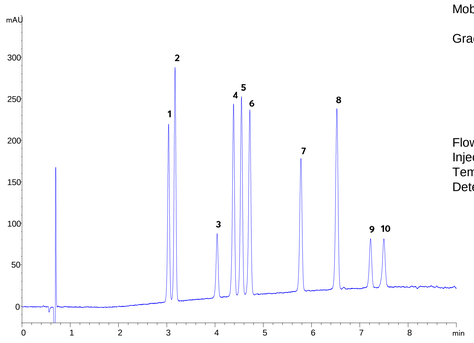
Flow Rate: 0.6 mL/min
Injection: 5 µL
Temperature: 40 °C
Detection: UV, 210 nm

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. 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:

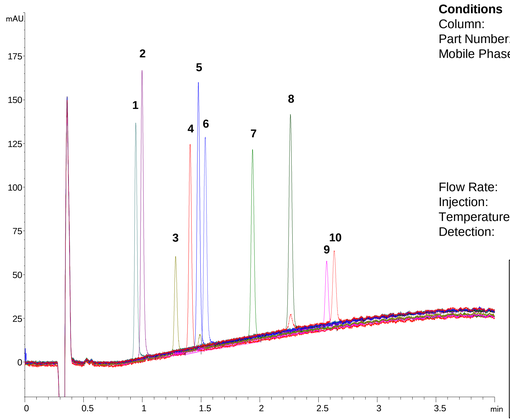
Time (mins)	%B
0.0	70
1.0	70
10.0	100
12.0	100

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. 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

Time (mins)	%B
0.00	70
0.25	70
4.18	100
5.05	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. 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 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.