

HPLC Application Note

Cannabinoids



HPLC Separation of 11 Cannabinoids on YMC-Triart C18

Jeffrey A. Kakaley, YMC America Inc.

This application note investigates using YMC-Triart C18 for HPLC potency analysis of 11 common cannabinoid compounds.

Background

In an effort to improve on YMC's original isocratic separation of seven cannabinoids on YMC-Triart C18, we set out to develop a method that can separate four additional minor cannabinoid compounds commonly encountered during potency testing. The original method utilized a 250x4.6mm, 5µm YMC-Triart C18 column and isocratic conditions, while the improved method uses a shorter (150mm) and smaller particle size (3µm) column with gradient separation.

The 11 cannabinoids analyzed:

- Cannabivarin (CBV)
- Cannabidiolic Acid (CBDA)
- Cannabigerol (CBG)
- Cannabidiol (CBD)
- Tetrahydrocannabivarin (THCV)
- Cannabinol (CBN)
- Delta-9-tetrahydrocannabinol (Δ^9 -THC)
- Delta-8-tetrahydrocannabinol (Δ^8 -THC)
- Cannabicyclol (CBL)
- Cannabichromene (CBC)
- Tetrahydrocannabinolic Acid (THCA)

Sample Preparation

Each cannabinoid standard came from the manufacturer as a 1.0mg/mL stock solution in either acetonitrile (ACN) or methanol (MeOH). 0.05mg/mL working samples of each cannabinoid were made by diluting each 1.0mg/mL stock solution using a diluent of 75:25 ACN:H₂O. This was performed by pipetting 50µL of each stock solution into a 2mL HPLC vial, followed by 950µL of diluent. A 0.05mg/mL mix of the 11 cannabinoids was made by adding 50µL of each stock solution to a 2mL HPLC vial followed by 450µL of diluent. All samples were mixed well via vortex mixer.

Operating Parameters

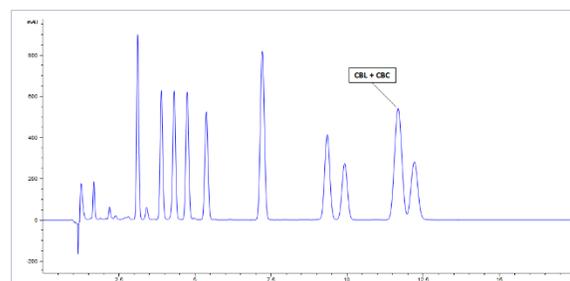
Mobile Phase A: HPLC water w/ 0.1% Formic Acid
Mobile Phase B: Acetonitrile w/ 0.1% Formic Acid
Column Temp: 35°C
Flow rate: 1.0mL/min
Inj. Volume: 10µL
Detection λ : 220nm
Column: YMC-Triart C18, 150x4.6mm, 3µm, P/N: TA12S03-1546WT
HPLC System: Agilent 1260
Gradient:

RT (min)	%A	%B	Flow (mL/min)
0.0	25.0	75.0	1.0
20.0	20.0	80.0	1.0
20.1	25.0	75.0	1.0
25.0	25.0	75.0	1.0

Results and Discussion

At the initial stage of method development, the original isocratic method (79:21 ACN:H₂O) developed for the 250x4.6mm Triart C18 column was evaluated, however two of the newly added cannabinoids (CBL and CBC) coeluted on the shorter 3µm, 150x4.6mm column. This separation can be seen in Figure 1:

Figure 1: YMC-Triart C18 – Isocratic



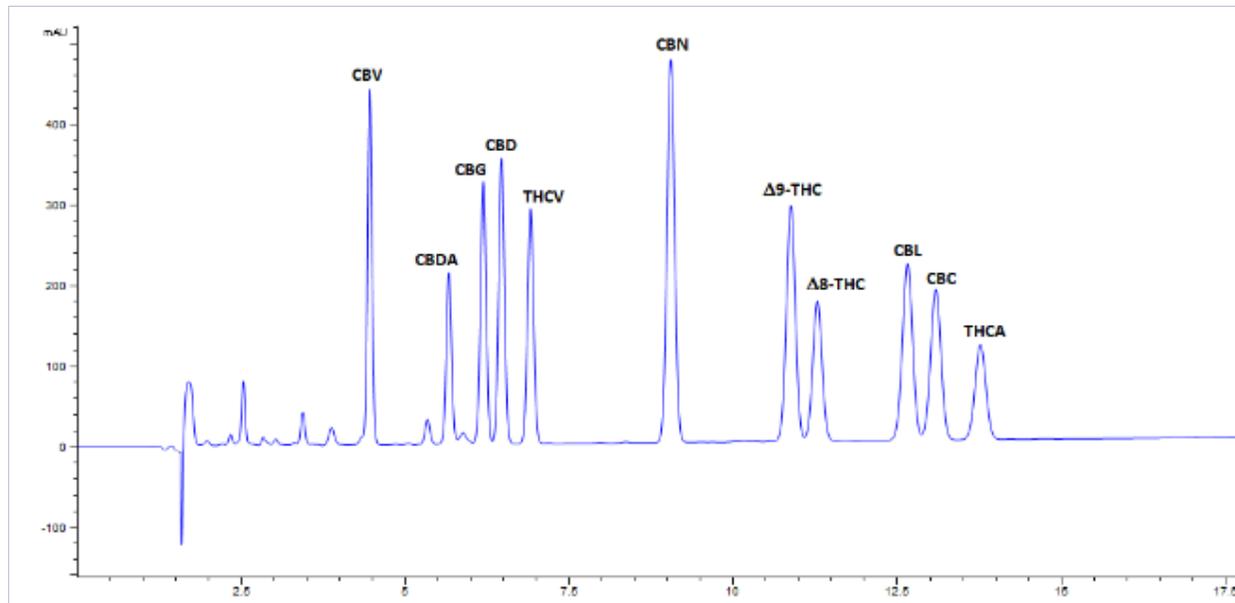
Other isocratic conditions were evaluated (75:25 and 70:30) but this only offered modest improvements in resolution and it was determined that a gradient method was necessary. Converting the method to gradient elution showed promise, and a shallow gradient of 75→80% ACN over 20 minutes provided acceptable resolution. The final separation can be seen in Figure 2:

HPLC Application Note

Cannabinoids



Figure 2: YMC-Triart C18 – Final Gradient



Conclusions

HPLC separations of cannabinoids can prove challenging due to the similar molecular structures and chemical properties of these compounds. Their hydrophobic nature allows for good retention on C18 columns, however good resolution can oftentimes prove to be elusive. As this application note indicates, YMC-Triart C18 provides the correct selectivity to separate 11 of the most common (major and minor) cannabinoids encountered during potency testing.

YMC America, Inc.
941 Marcon Blvd., Suite 201
Allentown, PA 18109
P: 610-266-8650
F: 610-266-8652
www.ymc-america.com