Analytical Cannabinoid Separations on Hybrid-Silica and Core-Shell Phases

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This application note investigates analytical HPLC potency testing of cannabinoids using YMC-Triart C18 and YMC Meteoric Core C18.

Sample Preparation

A 0.05 mg/mL standard mix of seven cannabinoids was made by pipetting 50 µL of each individual 1 mg/mL standard into a 2 mL HPLC vial and adding 650 µL of 50:50 acetonitrile–water. The 1 mg/mL cannabinoid standards used were: cannabidiolic acid (CBDA), cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), delta-8-tetrahydrocannabinol (Δ^8-THC), delta-9-tetrahydrocannabinol (Δ^9-THC), and tetrahydrocannabinolic acid (THCA).

Operating Parameters

Mobile phase:	79:21 acetonitrile–water
Column temp.:	35°C
Flow rate:	1.0 mL/min (hybrid-silica)
	1.25 mL/min (core-shell)
Inj. volume:	25 µL
Detection l:	220 nm
HPLC system:	Agilent 1260
Columns:	YMC-Triart C18, 250 x 4.6 mm, 5 µm, 120 Å
	YMC Meteoric Core, 100 x 4.6 mm, 2.7 µm, 80 Å

Results and Discussion

This application began as a customer inquiry requesting a fast and easy-to-run separation method for potency testing of seven specific cannabinoids. YMC Triart C18 was evaluated first because of the ability to scale the separation to different particle sizes for UHPLC as well as preparative separations. Several ratios of the acetonitrile–water mobile phase were screened before settling on 79:21. The separation using these conditions is seen in Figure 1.

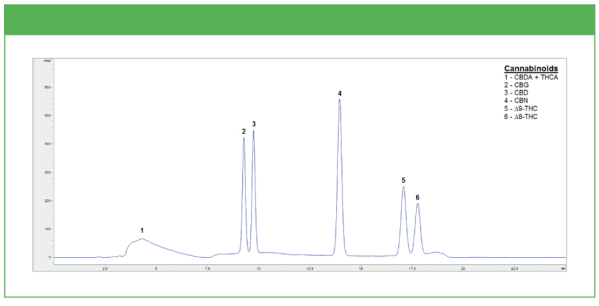

Figure 1: YMC-Triart C18—no acid.

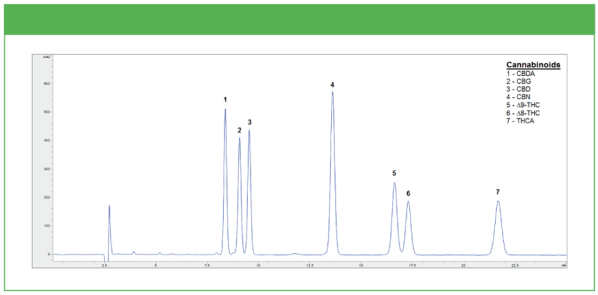

Figure 2: YMC Triart C18—0.1% formic acid.

The two acidic cannabinoids CBDA and THCA exhibited poor peak shape and retention under these conditions due to the lack of pH control in the mobile phase. The pH was lowered using formic acid to fully protonate both acidic species and gain retention, as can be seen in Figure 2.

Once the pH of the mobile phase was lowered, peak shape improved and retention times of both free-acid species increased dramatically, particularly in the case of THCA, which saw a retention increase of ~17 min.

The method was also tested on a YMC Meteoric Core C18 column to evaluate the speed of the core-shell material. Due to selectivity differences between the two stationary phases, the original mobile phase ratio was inadequate for use. Several ratios were evaluated and 73:27 acetonitrile–water (0.1% formic acid) was determined to give the best separation. The flow rate was also increased to 1.25 mL/min. The results can be seen in Figure 3.

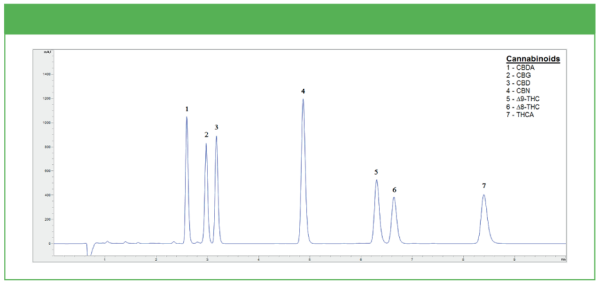

Figure 3: YMC Meteoric Core C18—0.1% formic acid.

Conclusions

The results indicate that the YMC Triart C18 and YMC Meteoric Core C18 stationary phases are good choices for analytical potency testing of these seven cannabinoids. These phases can also be used with low-toxicity mobile phases and additives such as ethanol–water and acetic acid for preparative purification. This was investigated using YMC-Triart C18 and YMC-Pack ODS-A with promising results (data not shown). Customers who are interested in this work are asked to please inquire.

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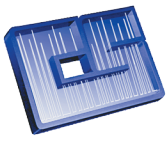

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