

# HPLC Application Note

## Cannabinoids



### HPLC Separation of Cannabidiol and Melatonin on YMC-Triart C18

Jeffrey A. Kakaley, YMC America Inc.

**This application note serves as a proof-of-concept HPLC method for cannabidiol (CBD) and melatonin.**

#### Background

At the request of a customer, YMC America, Inc. was asked to develop a quick and easy-to-run HPLC method for testing a mix of CBD and melatonin at a ratio of 5:1, respectively. The customer was interested in developing an over-the-counter (OTC) sleep aid that included both compounds as active ingredients and needed a simplified method for quantitative analysis.

#### Sample Preparation

A 1.0mg/mL stock solution of melatonin was made by massing out 10mg of melatonin into a 10mL volumetric flask. 5mL of diluent (45:55 acetonitrile:water) was added and the flask was then sonicated for 1 minute. After the melatonin was completely dissolved, the flask was diluted to mark. A stock solution of CBD did not need to be made as the standard itself came from the manufacturer as a 1.0mg/mL solution in acetonitrile.

A working sample was made in a 5:1 ratio of CBD:melatonin at concentrations of 0.05mg/mL and 0.01mg/mL, respectively. This was performed by pipetting 50µL of the CBD stock solution into a 2mL HPLC vial, followed by 10µL of the melatonin stock solution and 940µL of diluent, mixing well.

#### Operating Parameters

Mobile Phase A: HPLC water  
Mobile Phase B: Acetonitrile  
Column Temp: 40°C  
Flow rate: 1.0mL/min  
Inj. Volume: 15µL  
Detection λ: 225nm  
Column: YMC-Triart C18, 150x4.6mm, 3µm, P/N: TA12S03-1546WT  
HPLC System: Agilent 1260

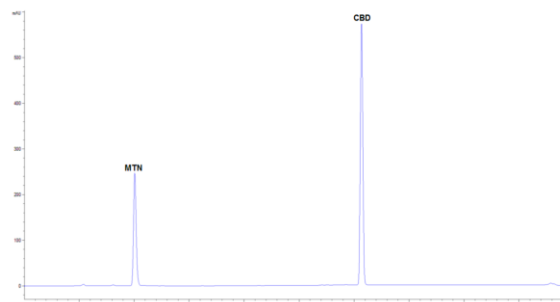
Gradient:

RT (min)	%A	%B	Flow (mL/min)
0.0	45	55	1.0
1.0	45	55	1.0
3.0	5	95	1.0
7.0	5	95	1.0
7.5	45	55	1.0
10.0	45	55	1.0

#### Results and Discussion

YMC-Triart C18 was the column chosen for this work due to its robust performance as a method development column. A number of isocratic conditions were evaluated early on, but poor peak shape of melatonin and lengthy elution time of CBD determined that isocratic conditions would not be ideal. A few different gradient conditions were attempted before settling on a 2 minute linear gradient (55→95%B) followed by a 4 minute hold at 95%B to elute CBD. The final separation can be seen in Figure 1:

**Figure 1: YMC-Triart C18 – Melatonin and CBD**



#### Conclusions

The results suggest that YMC Triart C18 is a good column candidate for separations involving CBD and melatonin. The goal of a fast and simple method with a runtime of < 10 minutes was accomplished to customer specifications. Depending on sample type and formulation, additional development work will most likely be necessary.

**YMC AMERICA, INC.**

941 Marcon Blvd., Suite 201  
Allentown, PA 18109  
P: 610-266-8650  
F: 610-266-8652  
www.ymcamerica.com