

#### TECHNICAL REPORT: AMT-TR062222

# TITLE: CANNABINOID SEPARATIONS AND THE IMPACT OF LC COLUMN SELECTIVITY

MARKET SEGMENT: CANNABIS

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# ABSTRACT

The natural products of the cannabis plant can number up to 100, but the scientific community is concerned with a smaller portion of around 10 to 20 compounds. Of these, the compounds of high interest are delta-8-tetrahydrocannabinol, delta-9-tetrahydrocannabinol, tetrahydrocannabinolic acid, cannabidiol, and cannabigerol. Separation of these compounds can be challenging, due to the large library of cannabinoids obtained from the plant, and requires the use of low pH mobile phases. These low pH mobile phases can reduce column lifetimes significantly if the column is not stable for use at low pH. With a low pH stable C18 phase cannabinoid separations can be done without the worry of stationary phase hydrolysis from acidic conditions. Having a choice of C18 columns with low pH stability that provide differing selectivity can also aid method development. Here we present a report on utilizing C18 chemistries for the separation of 16 and 18 cannabinoids.

# INTRODUCTION

Eight states have legalized marijuana for recreational use while even more states have allowed medical marijuana usage as of 2022. With the legalization of both medical and recreational cannabis across the United States there has been a surge in cannabinoid testing. Cannabinoids are a group of compounds that can be found in the cannabis plant. Some of these cannabinoid compounds are medically significant and can be used for pain management or appetite stimulation. It is important to test for these compounds to determine whether the products are safe for medical or recreational use. For potency testing the labs are concerned with specific compounds such as delta-8-tetrahydrocannabinol, delta-9-tetrahydrocannabinol acid (delta-9-THC), cannabidiol (CBD), cannabidiolic acid, and cannabigerol. Delta-9-THC and CBD are the two main components that were discovered early. These two compounds have opposite effects which makes them very important to quantify for potency. It is important to make the distinction between CBD and Delta-9-THC in medicinal testing due to the psycho-active properties of Delta-9-THC.

With more and more compounds of interest being added to the profile, chromatographers are in need for simple and reproducible methods to separate cannabinoids. The use of SPP particle technology is ideal for cannabis analysis due to the particles structural ability to perform high speed, high efficiency separations and be less prone to column clogging from dirty sample matrices.

With simple isocratic methods it can be difficult to achieve separation between all the cannabinoids of interest which means column choice becomes very important for the separation. Most cannabinoid methods require low pH mobile phases (pH of < 3) in order to obtain the separations required for potency testing. While C18 is

# **KEY WORDS:**

Cannabis, THC, Cannabinoids, Low pH, HALO 90 Å C18, HALO 90 Å LPH-C18, Column Selectivity

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a stable phase, the lifetime of the column will be reduced when operating routinely under low pH conditions. With the HALO® LPH- C18 phase, improved separations can be achieved along with a longer column lifetime. This increased lifetime can be attributed to the HALO® LPH-C18's sterically protected stationary phase. Many standard C18 phases do not contain this steric protection which leaves the stationary phase open to hydrolysis from the acidic conditions of low pH mobile phases.

### EXPERIMENTAL DATA:

All experiments were run using a Shimadzu Nexera HPLC system (Columbia, MD). The cannabinoid standards were acquired from Cerilliant (Round Rock, Texas). A HALO® 90 Å LPH-C18, 2.7µm, 4.6 x 150mm and a HALO® 90 Å C18, 2.7µm, 4.6 x 150mm column (Advanced Materials Technology, Wilmington, DE) were used as the analytical columns. Solvents and additives were obtained from MilliporeSigma (St. Louis, MO).

#### Cannabinoids:

Column: HALO® 90 Å LPH- C18, 2.7μm, 4.6 x 150mm, Part Number: 92824-716 Column: HALO® 90 Å C18, 2.7μm, 4.6 x 150mm, Part Number: 92814-702 Competitor Column: SPP 90 Å C18, 2.7μm, 4.6 x 150mm Mobile Phase A: 5mM Ammonium Formate in Water + 0.1% Formic Acid (pH = 3) Mobile Phase B: Acetonitrile + 0.1% Formic Acid Isocratic: 25/75 A/B Flow Rate: 1.5mL/min Back Pressure: 238 bar (LPH-C18), 247 bar (Standard C18) Temperature: 30 °C Detection: UV 228 nm, PDA Injection Volume: 1 μL Sample Solvent: 25/75 Water/Acetonitrile

# RESULTS (Mix of 16):

The first mix of 16 cannabinoids was prepared and injected on the system. The standard C18 column was used to create a controlled separation of the cannabinoids being tested. A total of 4 injections were performed to reproduce the results for each column. The comparison between the standard C18 and the LPH-C18 are in Figure 1.





As shown in the orange trace, the standard C18 exhibits more retention than the Low-pH C18 phase (grey trace) but the peaks for CBC and THCA-A (14, 15) coelute on the HALO® C18. This coelution is seen on the standard C18 due to the selectivity differences between both phases. The peaks for CBC and THCA-A are completely separated on the HALO® LPH-C18, low-pH phase along with all 14 other resolved peaks. Under these presented method conditions, the standard C18 cannot resolve peaks 14 and 15. It should be noted that with changes to the method conditions, the standard HALO® C18 column can separate all 16 compounds (AMT Application Note 222-CN) and offers rated stability to pH 2. Note two peak identities of I are CBCA degradation peaks.

## Results (Mix of 18):

The larger 18 mix contains two extra cannabinoids that could potentially be tested for in other facilities or labs. The two additional peaks are for the compounds exo-THC and CBLA. A low pH competitor SPP C18 column (grey trace) is unable to resolve the compounds CBNA and exo-THC while the HALO<sup>®</sup> LPH-C18 (orange trace), baseline resolves all of the compounds. Peaks 10 and 11 (exo-THC, CBNA) on the competitor column coelute almost to the point that the exo-THC peak could potentially be lost within the CBNA peak.



Figure 2. Isocratic separation of cannabinoids comparing AMT's HALO® LPH-C18 column and a competitor low pH SPP column. Peak Identities are Cannabidivarinic acid (CBDVA), Cannabidivarini (CBDV), Cannabidiolic acid (CBDA), Cannabigerolic acid (CBGA), Cannabigerol (CBG), Cannabidiol (CBD), Tetrahydrocannabivarini (THCV), Tetrahydrocannabivarinic acid (THCVA), Cannabinol (CBN), Cannabinolic acid (CBNA), exo-THC, Δ9-Tetrahydrocannabinol (Δ9-THC), Δ8-Tetrahydrocannabinol (Δ8-THC), Cannabicyclol (CBL), Cannabichromene (CBC), Tetrahydrocannabinolic acid A (THCA-A), Cannabichromenic acid (CBCA), and Cannabicyclolic acid (CBLA).

While the competitor C18 column cannot separate exo-THC, CBNA under these method conditions, AMT's HALO<sup>®</sup> LPH-C18 can readily separate both compounds with a baseline resolution of over 1.5.

#### CONCLUSION:

Cannabinoid separations are complex due to the multitude of compounds produced by the plant. Most cannabinoid separations must be run at low pH for good peak shape and resolution. Low pH methods can reduce the lifetime of most standard C18 columns so having columns capable of performing well at low pH are advantageous. With the extra stability of the new HALO<sup>®</sup> LPH-C18 phase from Advanced Materials Technology, chromatographers can reliably perform low pH methods down to pH 1 with reproducibility and long lifetimes.

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