THC Remediation from CBD Extract to Demonstrate Running Flash Chromatography on a Prep HPLC Instrument

Dr. J Preston Ph.D. and Sean Orlowicz | Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501, USA

Introduction

Flash chromatography is often used for quick purifications during intermediate steps of pharmaceutical product synthesis. These intermediate purifications allow for higher yields of subsequent steps and ultimately more end product. Unlike LC columns, flash cartridges are considered disposable and as a result require less cleaning and maintenance since they are often disposed of after 1-3 purification runs.

The 2018 Farm Bill (the Agriculture Improvement Act of 2018) legalized the production and sale of hemp and its extracts in the USA. Currently, these hemp extracts are not considered illegal if they contain less than 0.3% THC (tetrahydrocannabinol). Some production techniques can produce hemp extracts that exceed this THC threshold and therefore, it will be considered a schedule 1 drug by the Drug Enforcement Administration. Flash chromatography is a potential technique that can be used to reduce the THC content and keep the extract below the 0.3% THC threshold.

When compared to Prep HPLC instruments, Flash instruments typically have basic functions, including low pressure pumps, a fraction collector and a UV-VIS detector. This simple setup allows for easier maintenance and a lower purchase cost. Likewise, flash cartridges are available in many common stationary phase chemistries suitable for reversed phase, normal phase and even HILIC separations. The latest high-end Flash instruments have been equipped with pumps that reach higher pressures and include sophisticated detectors. These systems are capable of running Prep HPLC columns. A system that can run both Flash columns and HPLC columns is a very versatile tool for a Prep Chromatography Lab. In most cases, a lab that already has a Prep HPLC (not designed as a Flash Instrument) can use their system to run Flash Columns. The work presented here will describe the adjustments necessary to run Flash columns on any typical Prep HPLC system in order to gain this extra level of versatility.

Instrumentation for Flash Chromatography

Traditional flash chromatography instruments

contain a low pressure pump, column holder, basic UV detector and fraction collector. (Ex. CHEETAH™ MP Series Flash Chromatography system)

High pressure flash instruments

have been commercialized in the last 10 to 15 years and are capable of running both Prep HPLC columns and Flash Chomatograhy columns. They utilize a pump that is capable of higher pressures, valves to switch between flash and prep columns, improved UV and other types of detectors, along with a fraction collector. (Ex. OCTOPUS Purification system)

Prep HPLC systems

have been around for many years. They have always had the capabilities of the recently available high pressure flash systems. The main difference is the fittings used for prep HPLC columns and flash columns. If we could use suitable adapters and fittings, we can use a prep HPLC system to run flash columns.

Comparing the low pressure flash, high pressure flash/prep and prep instruments above, all of the systems have a pump, detector and **fraction collector. If a fraction collector is added to an analytical HPLC system**, we could use it to run flash or prep chromatography. The maximum flow rate is the most significant limitation.

Caution About Pressure

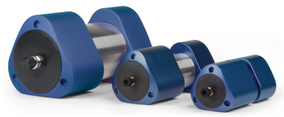
Another significant difference between prep HPLC and flash columns is the pressure limitations. Prep HPLC columns are designed for high pressure applications. The typical pressure limits for steel prep HPLC columns can range between 100 bar and 400 bar. Flash chromatography columns are a low pressure technique and these columns are not designed for high pressures. The typical pressure limit for plastic flash columns is usually below 20 bar.

The initial instruments designed for flash chromatography had pumps that could only function in the pressure range suitable for flash columns. The recent instruments (capable of using prep HPLC columns) are capable of over pressuring the flash column. If an HPLC system (prep or even analytical) was used with a flash column, over pressuring the column must also be a concern.

The Luer fittings on flash columns are typically rated to 35 bar. A Luer fitting can fail due to pressure as a partial leak or a total separation of the male and female Luer components. HPLC systems are designed with safety features such as leak sensors and low pressure limit settings that can turn off the eluent flow when a Luer fitting fails. Some individual Luer fittings, when assembled, can hold substantially more than 35 bar. If this happens and a high pressure instrument is being used, the plastic flash column body could be subjected to pressure much higher than their rated pressure limit. When the flash column body fails due to pressure, there could be a leak at the seal on the inlet side where the column is closed or the body could rupture. The leaking body is easily handled but the ruptured body could be a significant safety issue and should be addressed.

Fittings

The main differences between prep HPLC and Flash chromatography columns is the type of fittings used for the column inlet and outlet. Prep columns use high pressure fittings in combination with either 1/16" or 1/8" stainless steel or PEEK tubing. Flash columns use Luer fittings, with the inlet typically being a Luer lock fitting and the outlet being either a Luer/ Threaded or Snap lock fitting.



There are fittings and adapters available that make Luer fittings work with either 1/16" or 1/8" high pressure tubing. There are other fittings and adapters available that makes low pressure tubing work with high pressure fittings.

Outlet connection

Part # P-658

Luer Adapter 1/4-28 Female to Female Luer, PEEK

OR

Part # P-659

Luer Adapter 10-32 Female to Female Luer, PEEK

Intlet connection

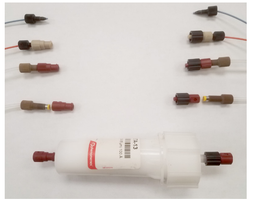
Part # P-655

Luer Adapter Assembly 1/4-28 Female to Male, PEEK

OR

Part # P-655

Luer Adapter 10-32 Female to Male, PEEK



Materials and Methods

Reagents and Chemicals

All chemicals and reagents were obtained from the Sigma-Aldrich Company (St. Louis, MO, USA). Water purification via Sartorius® arium® Comfort II (Goettinger, Germany).

CBD Sample is a hemp extract provided by local commercial supplier. This material contained the maximum amount of THC allowed before it is considered a schedule 1 drug.

Instrument Descriptions

CHEETAH™ MP Series Flash Chromatography System, Bonna-Agela Technologies

OCTOPUS Purification System, Bonna-Agela Technologies

Shimadzu ® LC-20AP pump

Agilent ® 1100 HPLC system

Column Descriptions for Analytical, Prep, and Flash

Flash Column:

Column: Claricep™ Screw-on Spherical C18, 20-35 µm,100 Å, Flash Column
Dimensions: I-Series, 20 g/column
Part No.: SN-S0230020-0

HPLC Prep Column:

Column: Luna® 5 µm C18(2) 100 Å, LC Column
Dimensions: 100 x 21.2 mm, AXIA™ Packed
Part No.: 00D-4252-P0-AX

Conditions for Flash, Analytical, and Prep

Column: As listed above
Mobile Phase: Isocratic: 10% Water with 0.1% Acetic Acid / 90% Acetonitrile
Flow Rate: 20 mL/min (unless specified)
Injection Volume: 100 µL (unless specified)
Temperature: Ambient
Detector: UV @ 220 nm
Sample: Hemp Extract, 500 mg/mL in 1-1-1 Tetrahydrofuran-Acetonitrile-Water

Conditions for Fraction Analysis

Column: Kinetex® 2.6 µm C18 100 Å, LC Column
Dimension: 150 x 4.6 mm
Part No.: 00F-4462-E0
Mobile Phase: Isocratic: 24% Water with 0.1% Formic Acid / 76% Methanol
Flow Rate: 0.75 mL/min
Injection Volume: 5 µL
Temperature: Ambient
Detector: UV @ 228 nm
Sample: Fractions directly from the fraction collector

Scalability: Analytical, Prep, and Flash

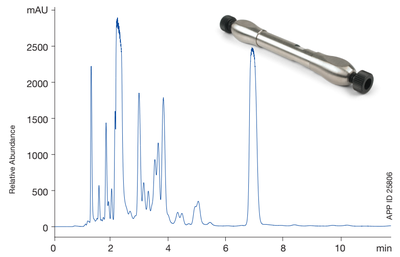


Figure 1: Chromatogram of CBD extract on analytical scale
Column: Luna® 5 µm C18(2) 100 Å
Dimension: 100 x 4.6 mm
Flow Rate: 1 mL/min
Injection Volume: 5 µL

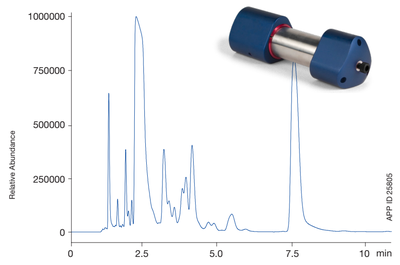


Figure 2: Chromatogram of CBD extract on prep scale
Column: Luna® 5 µm C18(2) 100 Å
Dimension: 100 x 21.2 mm
Flow Rate: 20 mL/min
Injection Volume: 20 µL

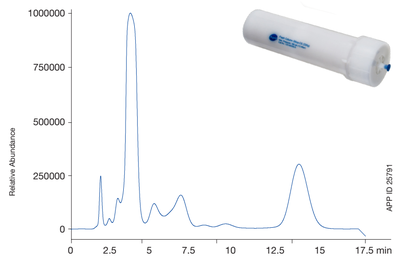


Figure 3: Chromatogram of CBD extract on flash
Column: Claricep Screw-on Spherical C18, 20-35 µm,100 Å, Flash Column
Dimension: I-series, 20 g/column
Flow Rate: 20 mL/min
Injection Volume: 100 µL

Results and Discussion

One of the best features of chromatography is scalability. Provided the instrumentation and column characteristics can be maintained, chromatographic performance can be scaled from small diameter columns to larger and larger columns. This principle is demonstrated here by scaling a CBD extract sample from a 4.6 mm ID diameter analytical scale to a 21.2 mm ID diameter prep scale. See **Figure 1** and **Figure 2**. The principle of scaling can be applied to Flash chromatography as well, but the differences in column characteristics will cause changes in the performance. See **Figure 1** and **Figure 3**.

In this case of THC removal from a CBD sample, a flash column easily provides enough resolution to perform this goal. See **Figure 5** and **Figure 6**. The Axia packed prep column provides chromatography that is essentially equivalent to the analytical column. With the prep column, 13 fractions were collected and the components were isolated as significantly pure fractions. See **Figure 4** and **Figure 5**.

Fractions collected from Axia

From a single injection of the crude sample run on an Axia prep column, 13 different fractions were collected. See **Figure 4**. Each fraction was evaluated with analytical scale HPLC methodology. See **Figure 5**.

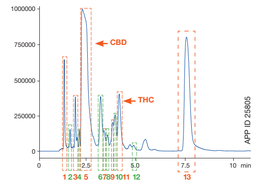


Figure 4: Chromatogram showing fraction collection
Column: From CBD extract on 100 x 21.2 mm Axia prep column
Dimension: 100 x 4.6 mm
Flow Rate: 20 mL/min
Injection Volume: 100 µL

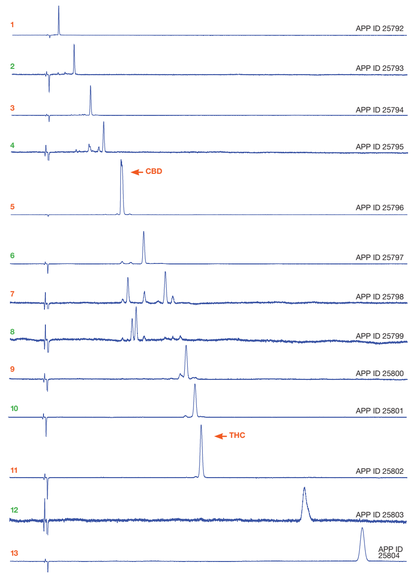


Figure 5: Chromatograms of collected fractions
Column: From CBD extract on 100 x 21.2 mm Axia prep column
Flow Rate: 20 mL/min
Injection Volume: 100 µL

Fractions collected from Flash

From a single injection of the crude sample run on a flash column, 4 different fractions were collected. See **Figure 6**. Each fraction was evaluated with analytical scale HPLC methodology. See **Figure 7**.

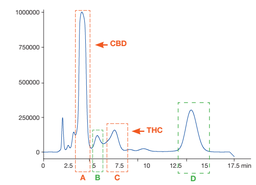


Figure 6: Chromatogram showing fraction collection
Column: From CBD extract on 20 g Flash column
Flow Rate: 20 mL/min
Injection Volume: 100 µL

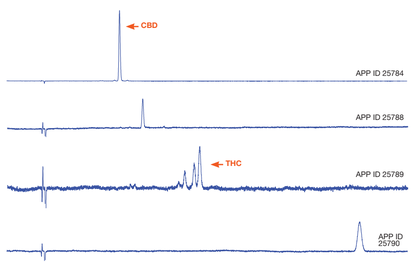


Figure 7: Chromatograms of collected fractions
Column: From CBD extract on 100 x 21.2 mm Axia prep column
Flow Rate: 20 mL/min
Injection Volume: 100 µL

Conclusion

Any chromatography system that can pump eluent can be used with Flash Chromatography columns. There are fittings and adapters that can connect the different types of tubing and components. This allows for greater versatility. One word of caution is to be aware of the operating pressure when using plastic flash cartridges. Flash Chromatography can be very useful when the resolution of peaks is not very demanding. Prep Chromatography is very powerful. If a separation can be done at the analytical scale it can scaled to prep. Chromatography (HPLC or Flash) can be used for THC remediation from hemp extracts. This work was done with acetonitrile but the chromatography can easily be converted to another strong eluent such as ethanol.

Have questions or want more details? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

PO76069920_W