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PURE LABS

Does Syringe Filtration Impact Recovery of Cannabinoids Prior to Potency Testing?

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Abstract

To ensure consumer safety, cannabis and cannabis-derived products are extensively tested. In particular, potency testing to identify and quantify tetrahydrocannabinol (THC) and other cannabinoids, like cannabidiol (CBD), is mandatory. To protect analytical instrumentation during testing by HPLC, particulate components must be removed from the sample after extraction, for example using syringe filters.

The objective of this study was to determine if using syringe filters (Sartorius Minisart® RC, SRP, NY and NY Plus, all 0.2 µm) either released interfering compounds or retained cannabinoids during sample preparation for HPLC testing. We used a blank matrix specimen of homogenized nettle leaf (Urtica dioica) for calibrator and control and analyzed without cannabinoid for leachables. In addition, a true cannabis specimen was filtered using each of the four different filter types and analyzed for recovery of cannabinoids by HPLC.

The results indicate that the various syringe filter types did not have a significant impact on quantification of cannabinoids.

Introduction

Cannabis extracts from cannabis flowers or in cannabis-infused products, tend to have heavy particulates. Therefore, an essential element of sample preparation prior to analysis with HPLC is the removal of particles. These particulates that become deposited in the system can lead to wear of the pumps and valves or to unwanted fluctuations in pressure and, ultimately, to a reduction in quality of the results due to increased blocking of the columns and frits. Improper sample preparation results in increased downtime and maintenance efforts, and reduced cost-effectiveness.

Particulates can be removed from samples by centrifugation, filtration, or a combination of both. Filtration, usually by syringe filtration, offers the security of a complete removal of particles, even suspended matter, which cannot be separated satisfactorily by centrifugation due to the small difference in density compared to water.

However, with filtration, the test results can be affected by the contact between the sample and the membrane material. In particular, test results can be affected by adsorption of analytes at the membrane and leaching of interfering compounds from the filter membrane. Therefore, researchers must evaluate for such occurrences during sample preparation.

We tested four different 0.2 µm Sartorius Minisart® filters, differentiated by their membrane material (regenerated cellulose, PTFE, Nylon, and Nylon with a quartz pre-filter) to test for the presence of leachables and retention of cannabinoid analytes.

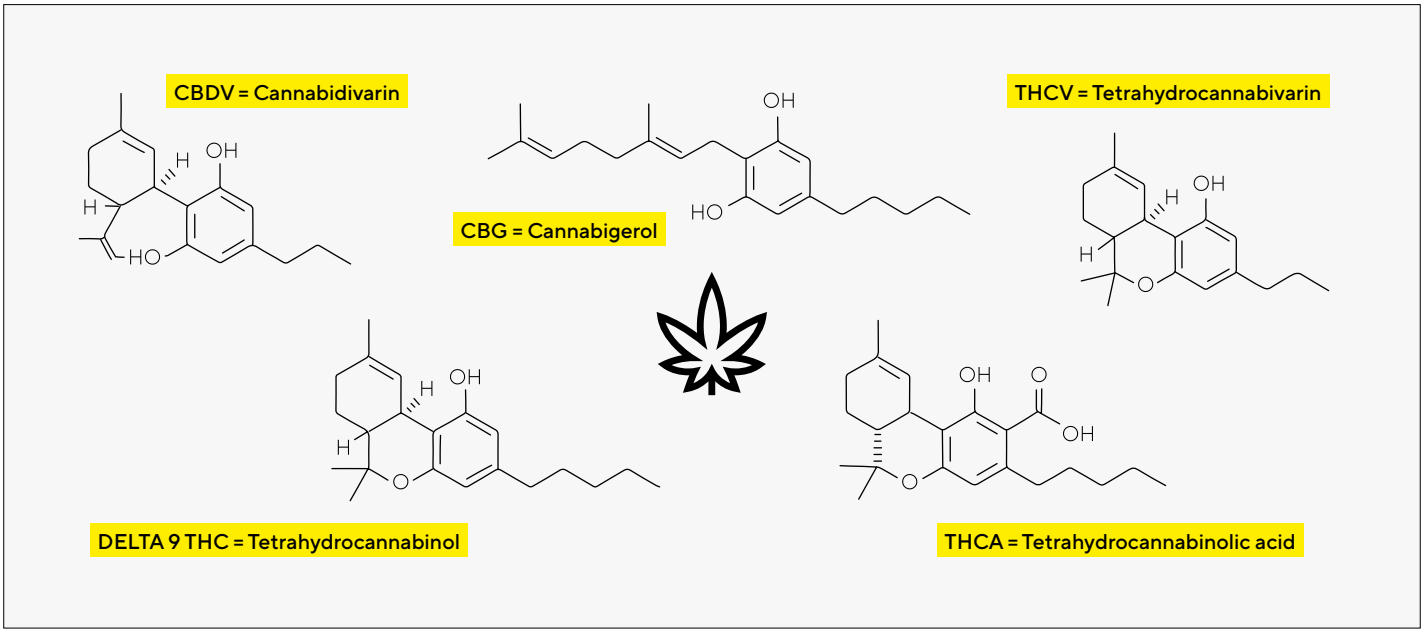

Figure 1: Cannabinoids used for determination of recovery.

Materials and Methods

Individual cannabinoid reference standards, as well as the internal standard (ISTD) phencyclidine (PCP), were purchased from Cerilliant (Round Rock, TX) as single substances. Four different Sartorius Minisart® filter types were tested:
Minisart® X Plus 25, 0.2 µm (1784B), Lot: 70007106,
Minisart® NY, 0.2 µm (17845) Lot: 81932103,
Minisart® RC, 0.2 µm (17764), Lot: 90129103 and
Minisart® SRP, 0.2 µm (17575) Lot: 90172103.

HPLC measurements were done with a Shimadzu Nexera-i LC2040L 3D Plus analyzer, fitted with a Restek Rapture ARC-18 (931421E) 100 mm × 3.0 mm ID × 1.80 µm column and coupled with a Restek UltraShield pre-column filter, 0.2 µm frit (25809) under temperature control at 30° C. The HPLC was operated in isocratic mode with 25 % aqueous (Water, 5 mM ammonium formate, 0.1 % formic acid) and 75 % organic (Acetonitrile, 0.1 % formic acid) phase at a flow rate of 1 mL/min. A sample volume of 1 µL was injected and with photodiode array detection at 190–400 nm wavelength.

A stock solution in methanol containing 17 cannabinoids and the internal standard (ISTD) at a concentration of 100 µg/mL was prepared. This stock solution was serially diluted and used as a calibrator. Additionally, a second stock

solution in methanol containing the same 17 cannabinoids was prepared and diluted for use as a positive control. A 4-constituent control prepared from an alternate reference standard source was included as a high control. To prepare a matrix solution, 20 mg of ground Stinging Nettle leaf *(Urtica dioica)* was taken and vortexed with 20 mLs methanol. Afterwards, the suspension was sonicated and vortexed, then centrifuged, filtered, and stored for preparation of calibrators, controls, and blanks.

Cannabis samples were prepared in the following way: one to two grams of cannabis was placed in a 50 mL centrifuge tube, a grinding ball added, and the tube frozen for 1 hour. This centrifuge tube was then placed on a Geno Grinder for 1 minute at 1600 rpms. 0.2 grams ground cannabis was removed and placed in separate 50 mL centrifuge tube with 20 mL methanol. Extraction was accomplished performed by cycling three times between vortexing for 1 minute and sonication for 5 minutes and repeating this extraction cycle three times. A 1 mL aliquot was removed and filtered, then ultra-centrifuged for 5 minutes at 14000 rpms. After filtering, 100 µL of undiluted and prepared diluted sample was added to a 150 µL auto-sampler vial, internal standard added, and analyzed on the HPLC.
A calibration curve, blanks, and controls were prepared to determine acceptance and tolerance limits. The cannabis specimens were evaluated against the linear dynamic range of the calibration curve, and reported in µg/mL.

A total of 110 samples were analyzed. All combinations were analyzed quantitatively with 10-fold repetition (Table 1). To check the filters for purity, blanks, positive controls, and filtrates of the blank solution using different syringe attachment filter types were analyzed. To calculate the recovery rate in percent of cannabinoids after filtration, unfiltered samples and their filtrates for the four different syringe filters were also analyzed. The standard deviation was used to distinguish significant differences.

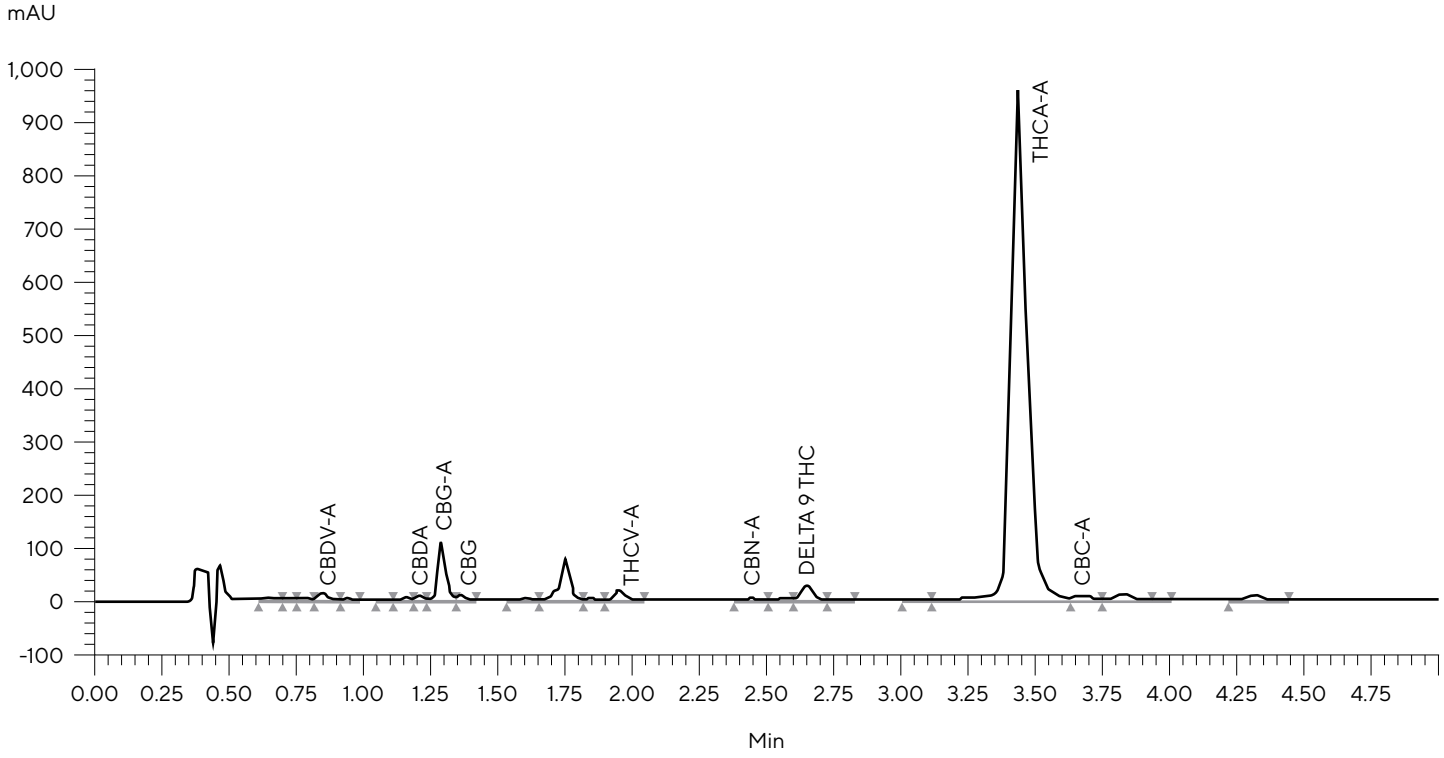

Figure 2: Cannabis samples were quantitatively analyzed using a set of 17 different cannabinoids. Only six analytes (CBDV-A, CBG-A, CBG, THCV-A, DELTA 9 THC, THCA-A) showed values above the LOQ for replicates and have been considered for calculation of cannabinoid recovery rates.

Number of Replicates	Positive Control	Blank UNFILTERED	Blank FILTERED	Sample UNFILTERED	Sample FILTERED
Minisart RC	10	10	10	10	10
Minisart SRP			10		10
Minisart NY			10		10
Minisart NY Plus			10		10

Table 1: Concept of experiments and overview of combinations conducted and their replicates to determine the presence of interfering leachables and cannabinoid recovery of four different syringe filter types. Recovery rate of cannabinoids is calculated by the ratio of 'Samples FILTERED' and 'Samples UNFILTERED'. Pureness is calculated by difference of 'Blank FILTERED' and 'Blank UNFILTERED'.

Results and Discussion

In this study, we examined four different syringe filter types to determine if they released interfering compounds into a blank sample. Figure 2 shows that no interfering compounds were detected in the blank sample, as compared to our positive controls (blank samples spiked with cannabinoids). While most analytes were spiked with a concentration 10 µg/mL, CBDA and CBD were spiked with 50 µg/mL and Delta 9 THC and Delta 8 THC are spiked with 75 µm/mL. All positive controls were within the expected range.

Additionally, we examined the recovery rate for cannabinoids using these syringe filters to determine these analytes were retained within the filter matrix. We defined the recovery rate as the ratio of filtered analytes to unfiltered analytes and expressed the ratio in percent. To calculate the recovery rate, we considered only quantifiable analytes. Figure 3 shows recovery rates ranging between 86.2 % and 126.5 % for all the syringe filters we tested. We found no significant reduction in analytes and no significant differences in recovery rate between filter types (Figure 4).

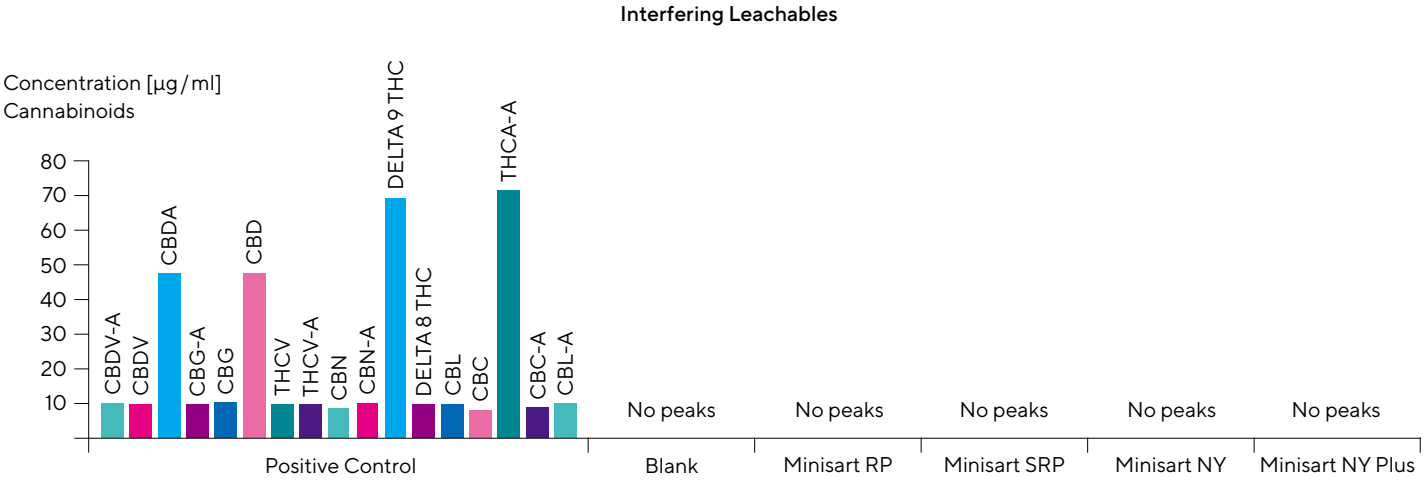

Figure 3: No interfering compounds were detected using any of the four syringe filters to remove particulates. We prepared a samples, including blank (Blank FILTERED) samples, using four types of syringe filters: Minisart RC, Minisart SRP, Minisart NY and Minisart NY Plus with a pore size of 0.2 µm. The samples were run over an HPLC column to determine if they released compounds that interfere with our quantification of 17 exemplary cannabinoids.

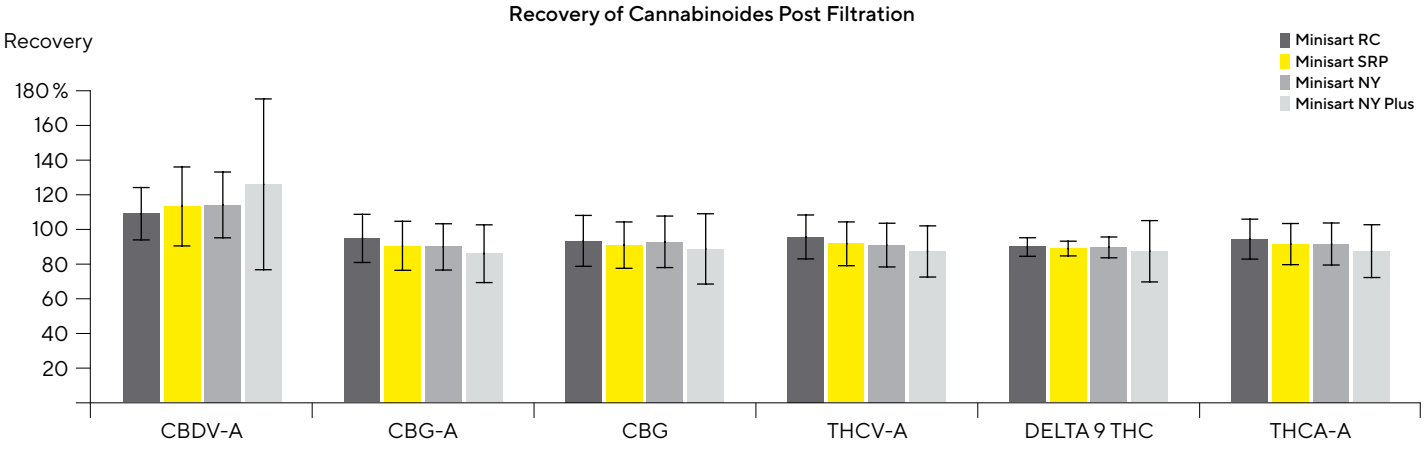

Figure 4: No significant reduction in analytes during filtration and no significant differences between syringe filter types. We examined the recovery rate of six different cannabinoids after filtration for four syringe filter types: Minisart RC, Minisart SRP, Minisart NY and Minisart NY Plus with a pore size of 0.2 µm.

Conclusion

In this study we tested four syringe filters types for their suitability in cannabis analysis by HPLC. Syringe filters tested should neither release interfering compounds nor retain cannabinoids. All investigated 0.2 µm syringe filters—Minisart RC (regenerated cellulose), Minisart NY (Nylon), Minisart NY Plus (Nylon incl. quartz micro fiber pre-filter) and Minisart SRP (PTFE)—showed no significant impact on the quantification of cannabinoids.

Finally, we conclude that all syringe filters tested support an accurate quantification of cannabinoids by HPLC. This outcome allows users to choose between different filter membrane materials for sample preparation according to their specific needs.

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