A Study of Filter Types Used in Sample Preparation of Cannabis/Hemp with HPLC Analysis

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Introduction

Filtration is a critical step in preparing cannabis and hemp samples for HPLC potency analysis. Fine particles must be removed to make the sample suitable for HPLC injection, following extraction into a suitable solvent. Syringe filters, while effective for particulate removal, can sometimes be problematic in terms of analyte adsorption, resulting in some loss of target recovery.

We conducted a study to determine the recovery of phytocannabinoids (in terms of concentration) using several syringe filters. Seven types of syringe filters were tested with methanol used as the solvent medium, as per the manufacturer's recommendation. Our goal was to determine the recovery (without pre-wetting) of phytocannabinoids using seven different types of filters; polyvinylidene difluoride (PVDF-hydrophobic), modified polyvinylidene difluoride (PVDF-hydrophilic), polypropylene (PP), polytetrafluoroethylene (PTFE-hydrophobic), nylon, cellulose acetate (CA) and polyether sulfone (PES).

Equipment and Method

For this study a Shimadzu Cannabis Analyzer for Potency™ – an integrated HPLC system with built-in UV detector – was used. We conducted a solvent spiking evaluation of the filters using methanol as the un-spiked, un-filtered solvent. Methanol was then spiked to 10 µg/ml (spiked, un-filtered solvent) using a 250 µg/ml cannabis standard. The calibration curve was built with the number of points as indicated in the Cannabis Analyzer for Potency™ high-sensitivity method using the 11-part phytocannabinoid mix (CRM; PN: 220-91239-21) in the prescribed solvents.

Quality Control (QC) standards were prepared using the same method as the calibration standards. Both QC standards were run before and after each filter type. Spiked, filtered solvent was pushed through the syringe filter in replicates of n=10, and a new filter was used each time. Processing was performed using each filter and a new filter each time, resulting in 10 individual preparations for each filter type (70 prepared) ready for injection using the Cannabis Analyzer for Potency™.

Table 1 Instrument and method parameters

Item	Details
HPLC System	Cannabis Analyzer for Potency™, 220-94420-00
Mobile Phase A	0.085% Phosphoric Acid in Water
Mobile Phase B	0.085% Phosphoric Acid in Acetonitrile
Method and Gradient Program	High Sensitivity Method. 70% B for 3 min; 70%-85% B over 4 min; 85%-95% B over 0.01 min; 95% B for 0.99 min; 95%-70% B over 0.01 min; 70% B for 1.99 min
Oven Temperature	35 °C
Injection Volume	5 µL
Flow Rate	1.6 mL/min
Detector and Wavelength	UV-Vis at 220 nm
Standard	Phytocannabinoid mixture 11 (CRM), 220-91239-21
Column	NexLeaf CBX for Potency, 2.7 um, 4.6 x 150 mm column, 220-91525-70
Guard Column	NexLeaf CBX Guard Column Cartridge, 220-91525-72
Syringe and Filter	Luer-Lock, 0.45 µm porosity, 13 mm diameter disk, 5mL, 220-97330-50

Results and Discussion

Initial Calibration

A series of six initial calibration standards over the range of 0.5 to 100 µg/mL (parts-per-million, ppm) and two Quality Control (QC) standards, one at 20 ppm and one at 80 ppm, were prepared. The calibration curve was evaluated using both correlation coefficient (r^2) from a linear regression. All calibration curves passed the high sensitivity method criteria ($r^2 \geq 0.999$). Figure 1 shows the calibration curves for all compounds.

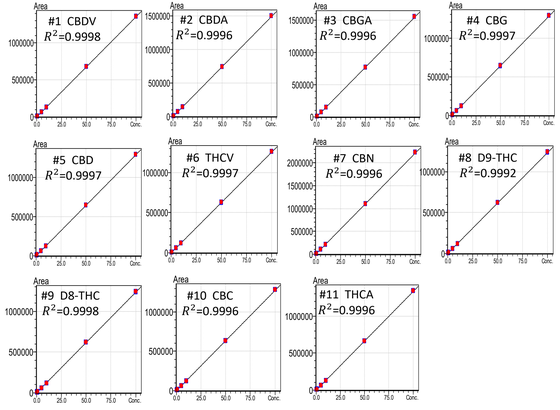

Fig. 1 Standard curves for 11 phytocannabinoids

Quality Control Standards

Quality Control (QC) standards with a concentration of 20 ppm and 80 ppm for all compounds were analyzed before and after each new filter type. The QC concentrations were calculated based on the initial calibration curve, and recoveries were within the calibration acceptance criteria. Table 2 shows the statistical results for the initial calibration curves and two representative QC standards. The statistical analysis was processed via Browser in LabSolutions Database, version 6.83; results are shown in table 2. Figure 2 shows a representative chromatogram of both the high and low QC standards. We conducted a spiked/un-spiked study to determine the percent recovery of the filters. We analyzed ten replicates of the spiked and un-spiked solvents, which were both un-filtered.

Table 2 Statistical results from the initial calibration and two representative QC standards

Compound	Standards (n=3)				Quality Control Standards			
					QC High (80 ppm)		QC High (80 ppm)	
	%Dev	Accuracy (%)	RF RSD (%)	R^2	%Dev	Accuracy (%)	%Dev	Accuracy (%)
CBDV	3.350	100.0	7.716	0.9998	2.420	102.3	1.680	99.0
CBDA	4.440	100.0	6.506	0.9997	2.490	102.4	2.330	97.8
CBGA	4.480	99.9	4.923	0.9997	2.480	102.4	2.530	97.5
CBG	4.120	99.9	4.171	0.9997	2.430	102.3	2.050	98.2
CBD	3.740	99.9	4.039	0.9997	2.600	102.6	1.970	98.4
THCV	3.710	100.0	7.272	0.9997	2.390	102.3	1.990	98.6
CBN	3.990	99.9	5.342	0.9997	2.500	102.5	2.190	98.2
d9-THC	15.630	99.9	30.944	0.9992	2.480	102.4	1.820	99.3
d8-THC	4.630	100.0	6.802	0.9998	2.760	102.7	1.730	99.5
CBC	4.800	99.9	6.746	0.9996	2.550	102.5	2.110	98.5
THCA	5.060	100.0	8.558	0.9996	2.370	102.4	2.270	97.9
Average	5.268	99.9	8.456	0.9996	2.497	102.4	2.061	98.5

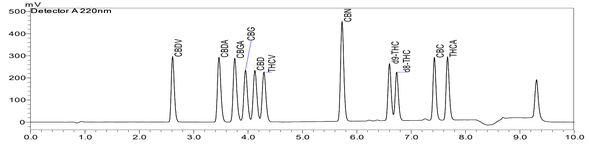

Fig. 2 A representative chromatogram showing separation of phytocannabinoids; QC high

Filtration Efficiency Study

A filtration efficiency study was conducted by analyzing 10 separate replicates per syringe filter containing a 10 ppm spike of 11 phytocannabinoids. Table 3 lists the details of the efficiency study. The results show that the nylon and PTFE syringe filters were the best candidates as they presented minimal hold-up of the phytocannabinoids and stable recoveries among ten replicates (nylon and PTFE sowed a %RSD of 0.82 and 0.87, respectively). Notably, we did not see a clear correlation between the hydrophilicity/hydrophobicity of the syringe filter's material properties and the level (or concentration) of the cannabis recovery. This implies that hydrophilicity does not impact filtration in a statistically significant manner. There were no significant differences in the recovered concentration of cannabis in filtered-spiked-solvent from unfiltered-spiked-solvent. These syringes should be considered as preferred for filtration of cannabis and hemp matrices.

Table 3 Filtration Efficiency Results

Compound	Syringe Filters (n=10)													
	CA		PP		Nylon		PES		PVDF- philic		PVDF-phobic		PTFE	
	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD
CBDV	11.19	6.481	10.17	2.558	9.70	0.608	10.90	3.207	10.88	2.050	9.97	1.382	10.01	0.913
CBDA	10.19	2.149	10.03	2.702	9.79	0.718	10.06	1.46	10.13	2.242	9.82	1.393	9.90	0.857
CBGA	9.85	1.932	9.98	2.708	9.58	0.704	9.66	1.244	9.77	2.152	9.64	1.911	9.86	0.814
CBG	9.83	2.025	10.07	2.747	9.57	0.555	9.72	1.491	9.79	2.288	9.79	1.568	9.93	0.851
CBD	9.82	2.058	10.08	2.645	9.61	0.669	9.70	1.672	9.76	2.052	9.81	1.706	9.94	0.919
THCV	9.81	2.242	10.10	2.514	9.63	0.675	9.73	1.697	9.77	2.175	9.83	1.644	9.93	0.725
CBN	10.21	2.403	10.09	2.819	9.59	0.843	10.07	1.756	10.05	1.800	9.89	1.394	9.93	1.217
d9-THC	10.18	2.192	10.21	2.622	9.61	0.768	10.03	1.994	10.07	2.196	9.95	1.358	9.96	0.692
d8-THC	10.33	2.462	10.27	2.405	9.78	1.330	10.19	1.492	10.17	2.694	10.08	1.454	10.18	1.075
CBC	10.12	2.561	10.16	2.916	9.70	0.923	9.93	1.904	10.05	2.374	9.95	2.079	9.96	0.802
THCA	10.10	2.573	10.13	3.459	9.72	1.214	9.99	1.932	10.03	2.386	9.88	2.679	10.01	0.759
Average	10.15	2.643	10.12	2.736	9.66	0.819	10.00	1.805	10.04	2.219	9.87	1.688	9.97	0.875

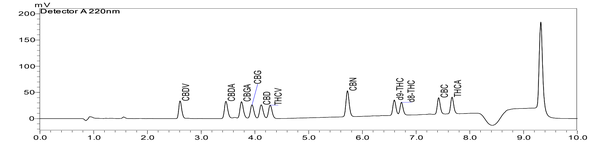

Fig. 3 Syringe filter evaluation. A representative chromatogram of the spiked filtered using nylon filter

Conclusion

We conducted a modified recovery study using syringe filters for sample preparation with the Cannabis Analyzer for Potency™. A quantitative HPLC method for the determination of 11 phytocannabinoids was used. Nylon and PTFE syringe filters were the best candidates as they presented minimal hold-up of the phytocannabinoids and stable recoveries among ten replicates (nylon and PTFE showed a %RSD of 0.82 and 0.87, respectively).