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Application News

No. CONS-001

Consumables / High Performance Liquid Chromatography

Sample Preparation of Cannabis Products by Syringe Filtration using the Shimadzu Cannabis Analyzer

■ Summary

Cannabis flower is an extremely complex matrix that must be filtered prior to HPLC or LCMS analysis to avoid clogging of the instrument. We evaluated syringe filters used during the sample preparation portion of the Cannabis Analyzer for Potency. The analysis was conducted using the Shimadzu Cannabis Analyzer for Potency High Sensitivity Method (CAP-HS). This analyzer is the most widely used HPLC instrument for determining the potency of cannabinoids found in Cannabis flower. Seven different syringe filter types were tested to determine which filter type was best for filtration of extracted cannabis flower. Three filter types were found to yield acceptable results but the Polypropylene (PP) filter was best.

■ Background

Many states across the United States are legalizing cannabis either for medicinal or recreational use. Laboratories testing this material are challenged with providing quality testing with varying reporting limits from state to state in an array of different matrices. This is why Shimadzu has developed three different methods for potency analysis of cannabinoids plus a standardized extraction method.

The extraction method currently being utilized for flower by many laboratories is a liquid-liquid extraction with filtration of the extract by using a 0.45um syringe filter prior to HPLC analysis.

■ Method

In this study, we evaluated the performance of the Shimadzu syringe filter products. Since extraction efficiency varies by laboratory and by analyst, we conducted both a solvent spiking and matrix spiking evaluation of the filters used in this method.

Using the extraction protocol provided with the Cannabis Analyzer for Potency, four representative cannabis flower samples were obtained, extracted, and homogenized to yield a final sample volume of 120mL. Additionally, Calibrators and Quality Control samples were prepared. Both QC samples were run before and after each filter type.

A 1mL aliquot of the sample was segregated as the unspiked unfiltered sample. The extracted sample was spiked using Shimadzu's 11-part cannabinoid mix (220-91239-21) to a concentration of 10ppm with a final volume of 100mL. 1mL of the spiked sample was segregated as the spiked unfiltered sample. 1 mL volumes of sample were filtered separately through each of the syringe filers in replicates of ten. The 0.45um porosity, 13mm diameter syringe filters were used for filtration.

Each sample, standard and QC was analyzed using the 10-minute CAP-HS method with UV detection. We accomplished near baseline resolution using a Shimadzu NexLeaf C18 2.7µ analytical column (220-91525-70) with associated guard column, as seen in the chromatogram in Figure 1.

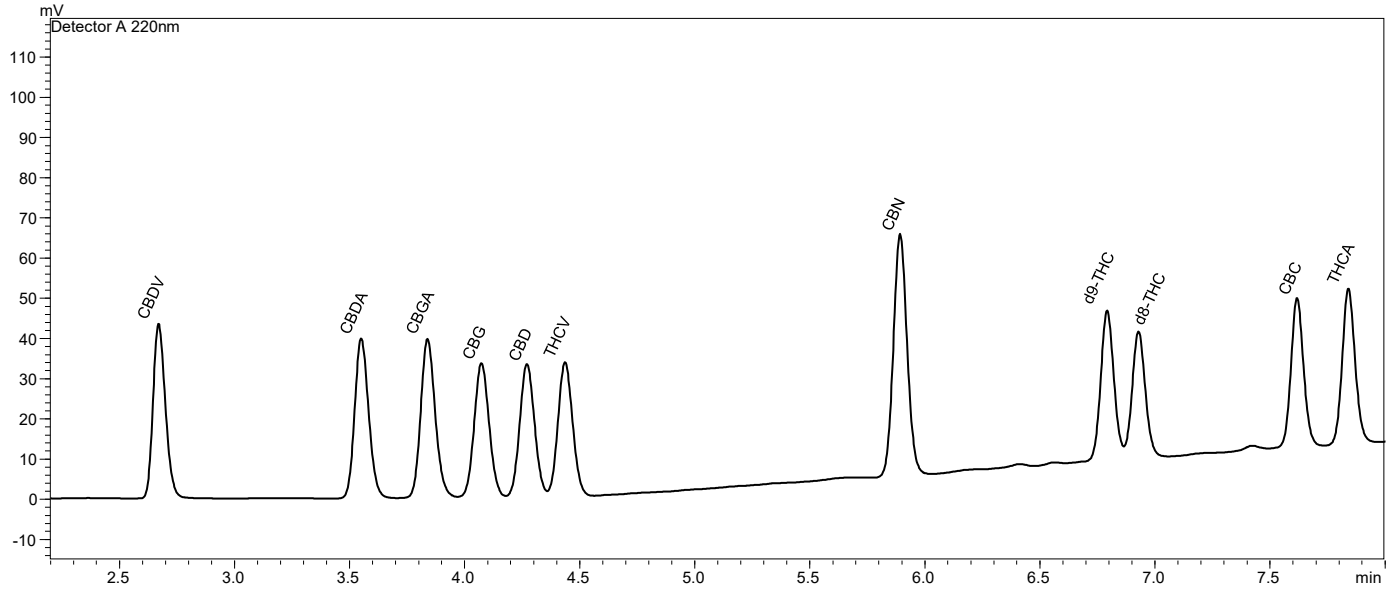

Figure 1: A representative chromatogram of the middle calibration point (10ppm) showing separation of all Cannabinoids.

■ Results and Discussion

Initial Calibration

A series of six initial calibration standards across the range of 0.5 to 100 ug/mL (parts-per-million, ppm) and two Quality Control (QC) samples, one at 20ppm and one 80ppm, were prepared. The calibration curve was evaluated using both correlation coefficient (r^2) from a linear regression and using the percent relative standard deviation (% RSD) for each data point in the curve. All calibration curves passed the CAP-HS criteria (RSD < 20%, $r^2 \geq 0.9900$). Figure 2 shows the calibration curves for all compounds.

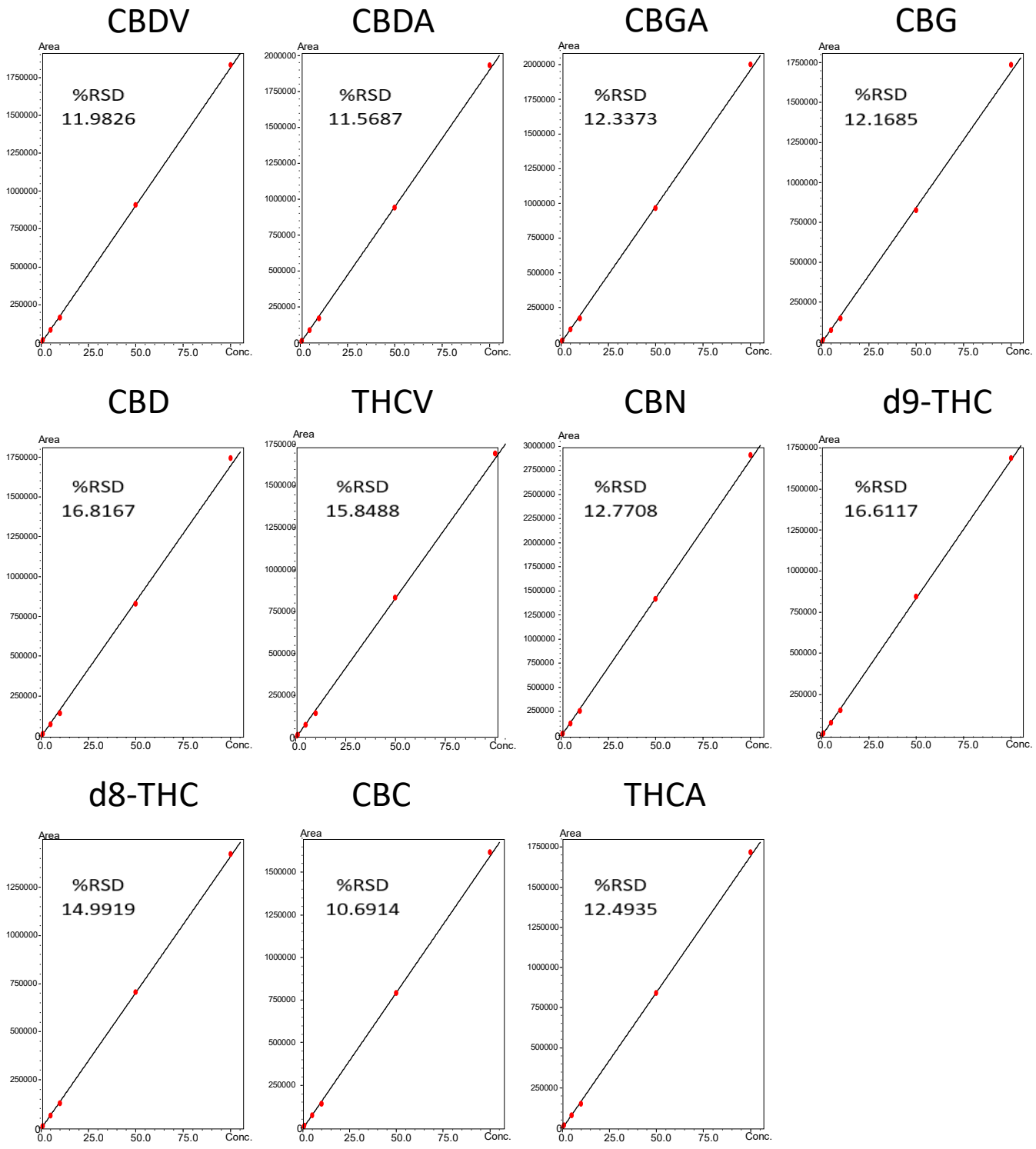

Figure 2: Calibration curves for all compounds contained in CAP-HS

Quality Control Standards

Quality Control (QC) standards with a concentration of 20ppm and 80ppm for all compounds were analyzed before and after each new filter type was tested. The QC concentrations were calculated based on the initial calibration curve, and recoveries were between 70 to 130% which is within the calibration acceptance criteria. Table 1 shows the statistical results for the initial calibration curves and two representative QCs. Figure 3 shows a representative chromatogram of both the high and low QC's.

Table 1: Statistical results from the Initial Calibration and two representative QCs.

6 Point Calibration Curve (n=3)								
Standards					OQ High		OQ Low	
Compound	**%Dev**	**Accuracy[%]**	**R^2**	**%RSD**	**%Dev**	**Accuracy[%]**	**%Dev**	**Accuracy[%]**
CBDV	5.010	100.011	0.999	11.983	2.850	97.100	13.32	86.70
CBDA	6.350	99.994	0.999	11.569	2.900	97.100	14.41	85.60
CBGA	6.610	100.000	0.999	12.337	2.530	97.500	14.83	85.20
CBG	6.890	100.000	0.998	12.169	1.670	98.500	15.23	84.80
CBD	9.260	99.994	0.997	16.817	1.460	98.700	14.97	85.00
THCV	8.390	99.989	0.998	15.849	2.160	97.900	13.56	86.40
CBN	7.410	99.994	0.999	12.771	2.910	97.100	14.91	85.10
d9-THC	5.410	99.994	0.999	16.612	2.820	97.200	12.92	87.10
d8-THC	6.450	100.011	0.999	14.992	3.170	96.800	13.28	86.70
CBC	5.970	99.994	0.999	10.691	2.950	97.100	14.32	85.70
THCA	5.330	99.994	0.999	12.494	3.060	96.900	14.19	85.80
verage	6.644	99.998	0.999	13.480	2.589	97.445	14.18	85.83

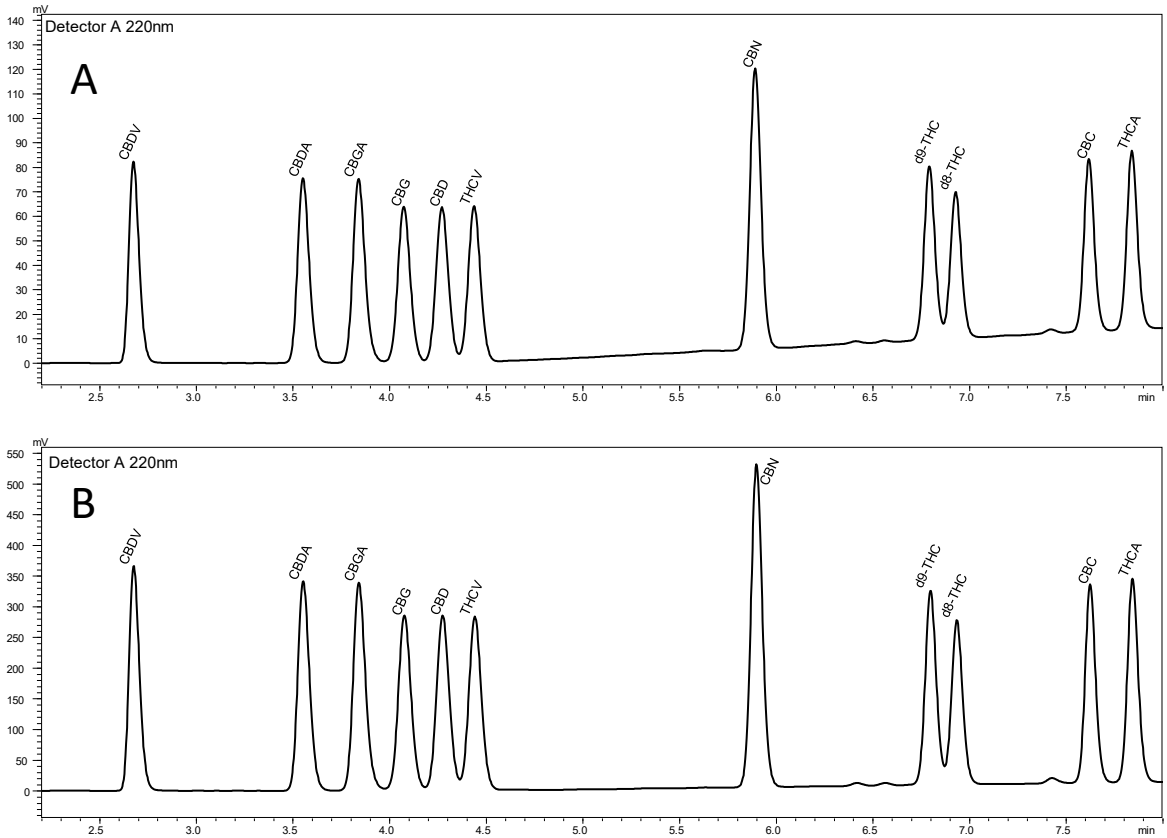

Figure 3: (A) Representative chromatogram of the QC Low (B) Representative chromatogram of the QC High

Unfiltered Spiked Samples

We conducted a Spiked-Unspiked study to determine the concentration of cannabinoids found in the natural product and to calculate percent recovery of the filters. We analyzed ten replicates of the spiked and unspiked samples which were both unfiltered.

Table 2 lists the detailed results of the study. The Spiked sample concentrations had %RSDs that were all less than 1 but the Unspiked sample had greater variability. The response remained stable during the entire study.

Filtration Efficiency Study

A filtration efficiency study was conducted by analyzing 10 separate replicate extractions per syringe filter type of cannabis flower that contained a 10ppm spike of 11 common cannabinoids. Table 3 lists the details of the extraction efficiency study. The Polypropylene, Nylon, and PTFE filters exhibited similar and the most stable recoveries while the PVDF-Hydrophilic had the most varying recoveries.

Table 2: Spiked vs Unspiked study results

Unfiltered Controls (n=10)				
	Spiked Unknown		Unspiked Unknown	
Compound	**Conc.**	**%RSD**	**Conc.**	**%RSD**
CBDV	6.833	0.853	0.503	1.567
CBDA	85.409	0.344	82.436	0.264
CBGA	29.435	0.383	23.740	0.216
CBG	20.409	0.406	14.440	0.446
CBD	71.471	0.285	66.903	2.284
THCV	10.914	0.610	3.274	17.929
CBN	21.118	0.274	14.914	0.517
d9-THC	553.181	0.331	562.499	0.307
d8-THC	No Data		0.653	1.992
CBC	23.790	0.993	17.710	0.382
THCA	542.939	0.466	567.935	0.389
Average	N/A	0.494	N/A	2.390

Table 3: Extraction Efficiency stability results.

	Syringe Filters n=10													
	CA		PP		Nylon		PES		PVDF-Hydrophilic		PVDF-Hydrophobic		PTFE	
Compound	**Conc.**	**%RSD**	**Conc.**	**%RSD**	**Conc.**	**%RSD**	**Conc.**	**%RSD**	**Conc.**	**%RSD**	**Conc.**	**%RSD**	**Conc.**	**%RSD**
CBDV	7.361	1.115	6.459	1.843	6.873	1.365	6.691	2.408	7.171	4.573	6.873	1.441	6.824	1.371
CBDA	90.740	0.968	78.568	1.048	86.390	1.280	82.635	2.525	88.741	4.795	86.747	1.514	86.051	1.197
CBGA	30.693	1.007	26.810	1.008	29.407	1.377	28.201	2.632	30.140	4.816	29.746	1.603	29.537	1.301
CBG	21.653	0.996	18.787	1.040	20.708	1.270	19.723	2.515	21.184	4.738	20.723	1.499	20.558	1.180
CBD	75.537	1.061	65.893	0.993	72.647	1.266	69.111	2.441	73.556	4.595	72.667	1.465	72.071	1.216
THCV	11.310	9.326	10.092	1.471	11.113	1.113	11.190	3.514	10.124	5.446	11.094	1.579	11.048	1.260
CBN	22.425	0.945	19.436	1.056	21.437	1.201	20.437	2.452	21.917	4.795	21.433	1.490	21.295	1.145
d9-THC	583.378	0.825	513.757	0.905	561.133	1.116	536.938	2.243	572.005	4.284	561.312	1.361	557.216	1.087
d8-THC	No Data													
CBC	25.429	1.017	21.794	1.128	24.097	1.426	23.240	2.738	24.713	4.771	24.153	1.668	23.956	1.419
THCA	586.854	1.388	492.804	1.172	551.859	1.619	521.850	2.961	571.490	6.196	553.829	1.884	548.325	1.533
Average	N/A	1.865	N/A	1.166	N/A	1.303	N/A	2.643	N/A	4.901	N/A	1.551	N/A	1.271

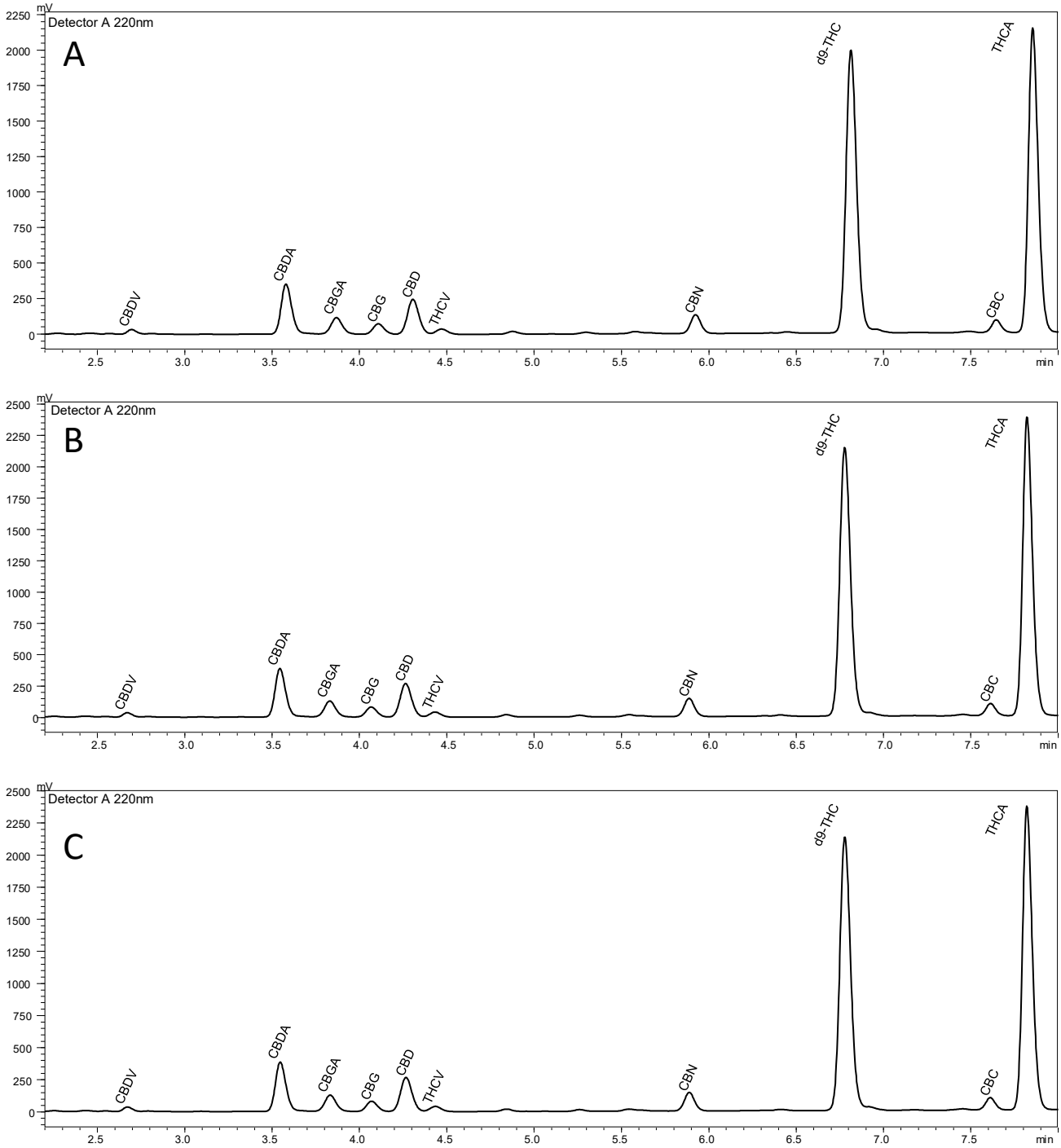

Figure 3: (A) Representative chromatogram of the Polypropylene filter (B) Representative chromatogram of the Nylon filter (C) Representative chromatogram of the PTFE filter

■ Summary and Conclusions

The Shimadzu polypropylene, nylon and PTFE syringe filters were determined to be the ideal filter types to use when conducting potency testing of cannabinoids in cannabis flower. The Polypropylene filter yielded the best results in terms of best recoveries, but is also the most expensive filter.

From a cost-effectiveness perspective, selecting a 0.45um porosity, 13mm diameter Nylon filter is the best choice because it had similar recoveries to the Polypropylene filter but at approximately half the cost.

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