RESTEK Pure Chromatography

A Streamlined, Cost-effective Cannabis and Hemp Flower Workflow for Potency Testing

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Introduction

As the cannabis industry sees continued growth, production labs are looking to improve sample throughput and cut costs per sample for potency analysis. Due to ever changing rules and regulations, it can become difficult to improve existing workflows. Keeping high-throughput labs in mind, a study was completed to obtain a streamline workflow with minimal process time, sample touch time, and to minimize the cost of materials required for sample preparation. The study was completed by comparing different extraction volumes, vortex times, and using a FilterMate (Environmental Express) versus centrifugation. A total of 16 cannabinoids were monitored on a UV-vis at 228 nm using a Raptor ARC-18 150 x 4.6 mm 2.7 µm analytical column with an accompanying 5 x 4.6 mm EXP guard under isocratic conditions, with a total cycle time of 10 minutes.

Experimental Design

This study assessed two types of filtration methods, the Environmental Express Digestion Tube with filter and plunger (FilterMate), and a standard 50 mL centrifuge tube followed by centrifugation and syringe filtration. Additional variables investigated were, two types of Environmental Express filters, 0.45 and 6 µm, extraction solvent volume, 10 and 20 mL, and vortex time, 5 and 10 minutes. All samples were assessed using 500 mg of flower. See Figure I for experiment flowchart.

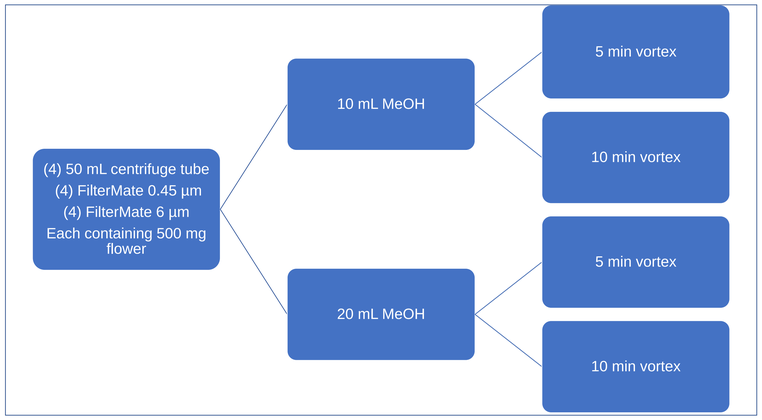

Figure I: Flowchart of experimental design

Sample Preparation

Method	Filtermate	Centrifuge
Filter size (µm)	0.45 and 6.0	0.22 (syringe filter)
Vortex Time (min)	5 and 10	
Solvent Volume (mL)	10 and 20	
Workflow	1. Weigh 500 mg into digestion tube 2. Measure required amount of methanol into digestion tube containing sample 3. Cap and place on multi- tube vortexer for designated time 4. Remove cap and add filter with hole facing up 5. Place plunger in hole and press down 6. Remove plunger and using pipet measure appropriate amount for dilution into amber vial 7. Dilute with 25:75 H2O:ACN and cap 8. Vortex briefly prior to analysis	1. Weigh 500 mg into 50 mL centrifuge tube 2. Measure required amount of methanol into centrifuge tube containing sample 3. Cap and place on multi- tube vortexer for 5 min 4. Centrifuge at 4000 RPM for required time 5. Using a plastic syringe, remove an aliquot 6. Filter sample into vial using syringe filter 7. Using pipet measure required amount into 2 mL amber vial 8. Dilute with 25:75 H2O:ACN and cap 9. Vortex briefly prior to analysis

Table I: Steps for workflow of each technique assessed

Cost and Time Analysis

To determine the cost of each consumable, an average using both high and low grade were calculated. The cost of each consumable was then added together to determine the final cost per sample.

Cost and Time Analysis (cont.)

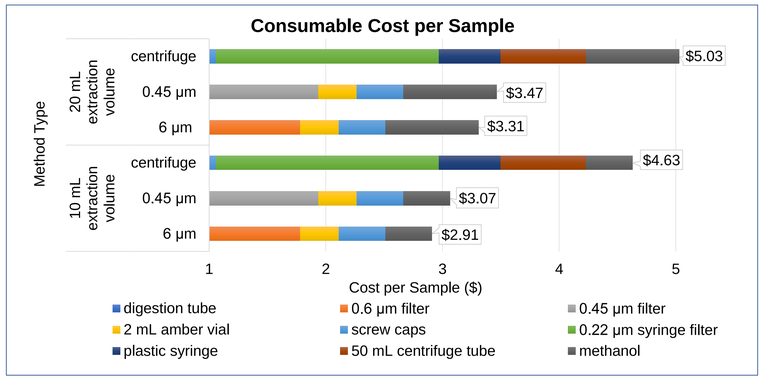

Figure II: Cost per sample for each analyzed method

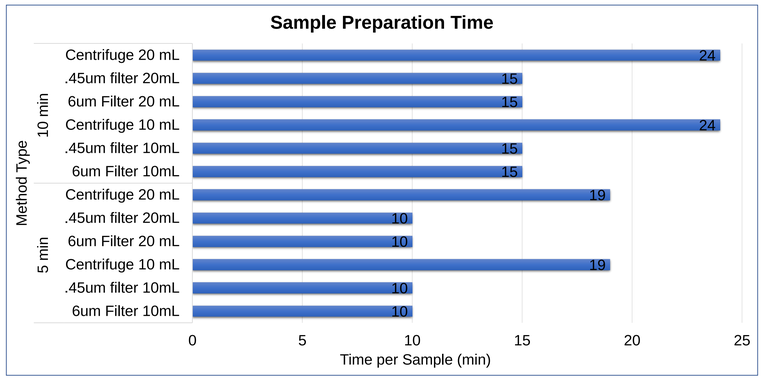

Figure III: Time per sample for each analyzed method

Analytical Method

Column:	Raptor ARC-18 2.7 µm 150 mm x 4.6 mm (Cat # 9314A65)	
Guard:	Raptor ARC-18 2.7 µm 5 mm x 4.6 mm ID (Cat # 9314A0250)	
Standards:	Acids 7 (Cat # 34144) and Neutrals 9 (Cat # 34132)	
Diluent:	25:75 Acetonitrile: Water	
Inj. Vol.	5 µL	
MP A:	Water, 5 mM ammonium formate, 0.1% formic acid	
MP B:	Acetonitrile, 0.1% formic acid	
Flow:	1.5 mL/min	
Detector:	UV-vis @ 228 nm	
Temperature:	30 °C	
Gradient:	Isocratic 25:75 MPA:MPB	
	Time (min)	(%) B
	0.00	75
	10.00	75

Table II: Method conditions for testing

Results

Results show that the use of a FilterMate outperforms that of a centrifugation workflow, in both cost and time, as shown in Figures II and III respectively. Data for all analytes is shown in Table IV. Table III indicates which workflow is the best for each category assessed.

Workflow Type	0.45 µm FilterMate	6 µm FilterMate	Centrifuge
Cost		✓	
Time	✓	✓	
5 min vortex time		✓	
10 min vortex time			✓
10 mL solvent			✓
20 mL solvent		✓	

Table III: Indicates which workflow performed best for each variable assessed

Solvent Volume	10 mL						20 mL					
Extraction Time	5 min			10 min			5 min			10 min		
Method Type	**0.45 µm**	**6 µm**	**Centrifuge**	**0.45 µm**	**6 µm**	**Centrifuge**	**0.45 µm**	**6 µm**	**Centrifuge**	**0.45 µm**	**6 µm**	**Centrifuge**
Cannabidivarinic Acid (CBDVA)	0.55	0.56	0.13	0.13	0.57	0.13	0.99	0.98	0.97	0.97	1.00	0.97
Cannabidiolic acid (CBDA)	15.79	15.51	15.77	15.79	15.51	15.77	26.76	26.67	26.56	26.29	26.86	26.50
Cannabigerolic acid (CBGA)	94.16	89.86	93.04	94.16	89.86	93.04	105.37	109.58	101.28	97.95	107.90	98.84
Cannabigerol (CBG)	12.17	11.60	34.05	12.17	13.10	34.05	7.35	41.55	13.78	7.57	41.79	52.29
Cannabidiol (CBD)	7.75	7.31	8.84	0.23	7.94	21.25	10.24	42.82	9.61	4.10	42.99	3.89
Tetrahydrocannabivarin (THCV)	ND	1.25	2.15	ND	ND	2.10	4.07	ND	0.61	4.25	4.47	0.61
Tetrahydrocannabivarinic acid (THCVA)	1.25	3.15	1.27	1.25	1.26	1.25	2.45	2.45	2.44	2.45	2.45	2.44
Cannabinol (CBN)	3.20	0.21	3.18	3.20	1.95	1.93	3.79	3.79	3.78	3.79	3.79	3.77
Cannabinolic acid (CBNA)	1.55	2.56	1.57	1.55	0.15	1.55	3.12	3.06	0.37	0.37	3.08	0.37
Δ9-Tetrahydrocannabinol (Δ9-THC)	2.58	2.56	2.64	2.58	2.60	2.56	4.95	4.92	4.90	4.69	4.69	4.69
Δ8-Tetrahydrocannabinol (Δ8-THC)	0.23	ND	ND	0.20	0.20	ND	0.42	ND	ND	ND	ND	ND
Cannabicyclol(CBL)	0.27	0.26	0.27	0.23	2.38	0.27	0.50	0.49	0.50	ND	0.49	0.49
Cannabichromene (CBC)	2.94	2.91	23.49	2.94	3.03	23.49	5.26	46.63	5.17	5.22	46.65	46.82
Tetrahydrocannabinolic acid a (THCA-A)	0.27	0.27	1.35	1.34	1.35	0.27	2.57	2.57	0.39	0.40	2.58	2.55
Cannabichromenic acid (CBCA)	2.14	2.08	2.24	2.14	2.18	2.05	3.77	3.79	0.99	3.71	3.87	3.63
Total THC:	2.82	2.80	3.82	3.76	3.79	2.79	7.21	7.18	5.24	5.04	6.95	6.92
Total CBD:	21.60	21.15	22.69	14.08	21.78	35.10	24.09	56.67	23.46	17.95	56.84	17.74
Total Cannabinoid Content:	144.85	140.08	189.98	137.92	142.08	199.69	181.61	289.31	171.33	161.76	292.63	247.86

Table IV: Concentration of each analyte in mg/g. Green and red indicators are highest and lowest results respectively.

Conclusions

By removing the centrifugation step, both the consumables cost, and the sample preparation time are effectively reduced, while still providing an efficient extraction of cannabinoids. The use of a 6 µm Environmental Express Tube, with 20 mL of solvent and 10 minutes of vortex time, prove to be the most cost effective and streamline workflow for flower.