Homogenization and downstream analysis of cannabis using the Omni Bead Ruptor 96 bead mill homogenizer.

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Omni Bead Ruptor 96 bead mill homogenizer

Introduction

Chronic pain is a growing epidemic and public health issue that continues to affect the aging population in the US. Managing pain is particularly difficult with patients who suffer from cancer-associated pain, neuropathic pain, as well as central pain states. These conditions are typically treated inadequately with opioids, antidepressants, and anticonvulsive drugs. There is growing evidence that cannabinoid natural products are beneficial for a range of clinical conditions, including pain and inflammation. The use of cannabinoids in highly-regulated prescription drugs is fast approaching, and the development of such drugs requires well-planned medical trials as well as very stringent guidelines for contaminants such as heavy metals, pesticides, mycotoxins, etc. Currently, each state manages their own set of regulations as they attempt to satisfy the growing demand for its products.

Here, we show how homogeneity is a critical process which can influence quantitation results of potency for certifying cannabinoid profiles of hemp flower and medicinal cannabis matrices. A fast and simple analytical approach which included homogenization and sample extraction to quantify 11 cannabinoids using an Agilent 1220 Infinity was performed. Analytical results obtained from cannabis samples were separated using HPLC techniques.

Using different parameters, we demonstrated the importance of homogenization in determining cannabinoid potency. During this experimental application the Omni Bead Ruptor 96 bead mill homogenizer successfully homogenized cannabis and generated reliable results for determining potency of 11 cannabinoids.

Materials and methods

- Omni Bead Ruptor 96 bead mill homogenizer (Cat# 27-0001)

- 50 mL Tube Holders (Cat # 27-1003)

- 50 mL Tubes with Screw Caps (Cat # 19-6650)

- Active Grinding Media (Cat # 19-900S)

Commercially available Hemp flower was purchased locally. 2 g of hemp flower samples were placed in a 50 mL tube (Cat # 19-6650) containing 1x Active Grinding Media (Cat # 19-900S). Samples were homogenized on the Omni Bead Ruptor 96 bead mill homogenizer using parameters outlined in Table 1.
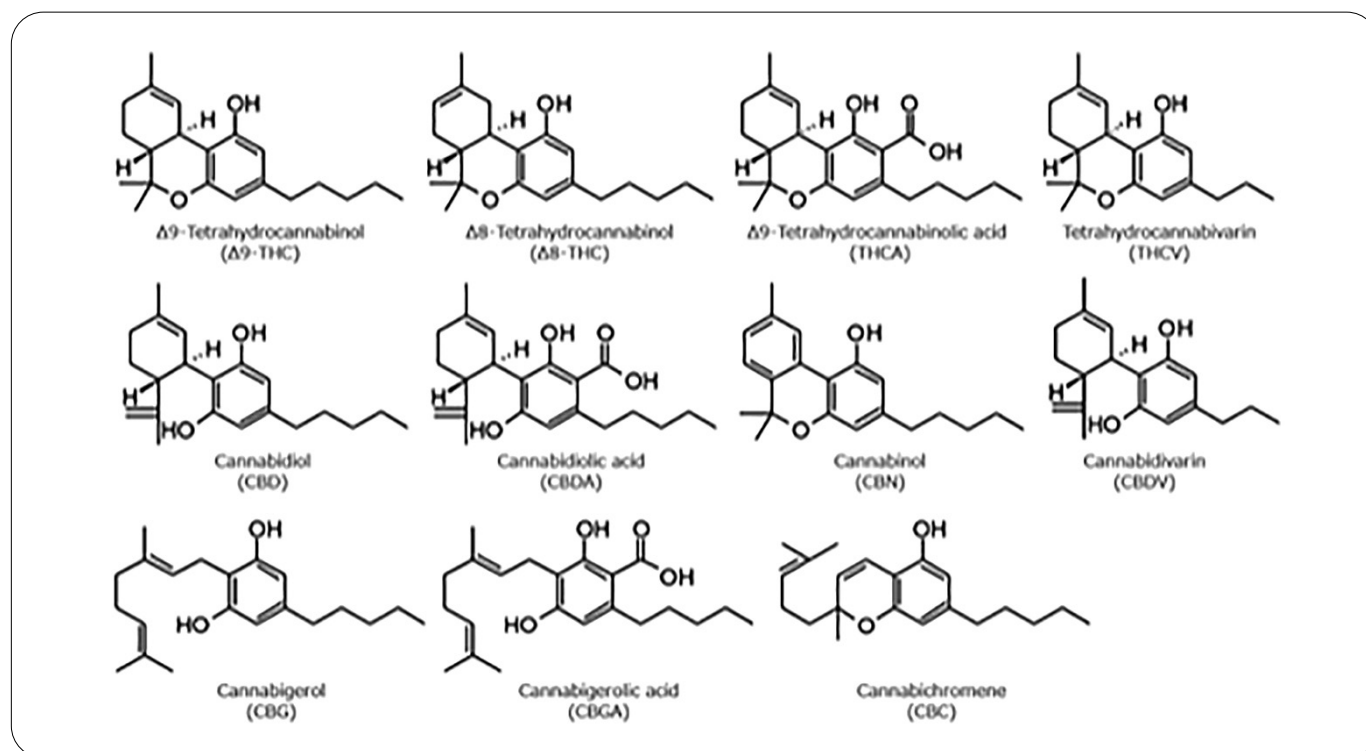

Figure 1: Main classes of natural cannabinoids in medical cannabis (marijuana) and hemp flowers, after homogenization, were identified in downstream analysis.

Table 1: Homogenization parameters of the Omni Bead Ruptor 96 bead mill homogenizer. All experimental conditions were carried out in triplicate using 2 g of Hemp flower.

Parameter	Frequency (Hz)	# of 15 sec cycles	Total homogenization time (sec)
Set 1	25	1	15
Set 2	25	2	30
Set 3	25	4	60
Set 4	25	6	90

Cannabinoid sample preparation method

Approximately 100 mg of homogenized flower matrix were extracted with 5 mL methanol. After sonication, vortexing, and centrifugation, an aliquot of the supernatant is diluted with MeOH/H_2O (80/20, v/v) + 0.1 % formic acid as appropriate for injection on an HPLC-UV.

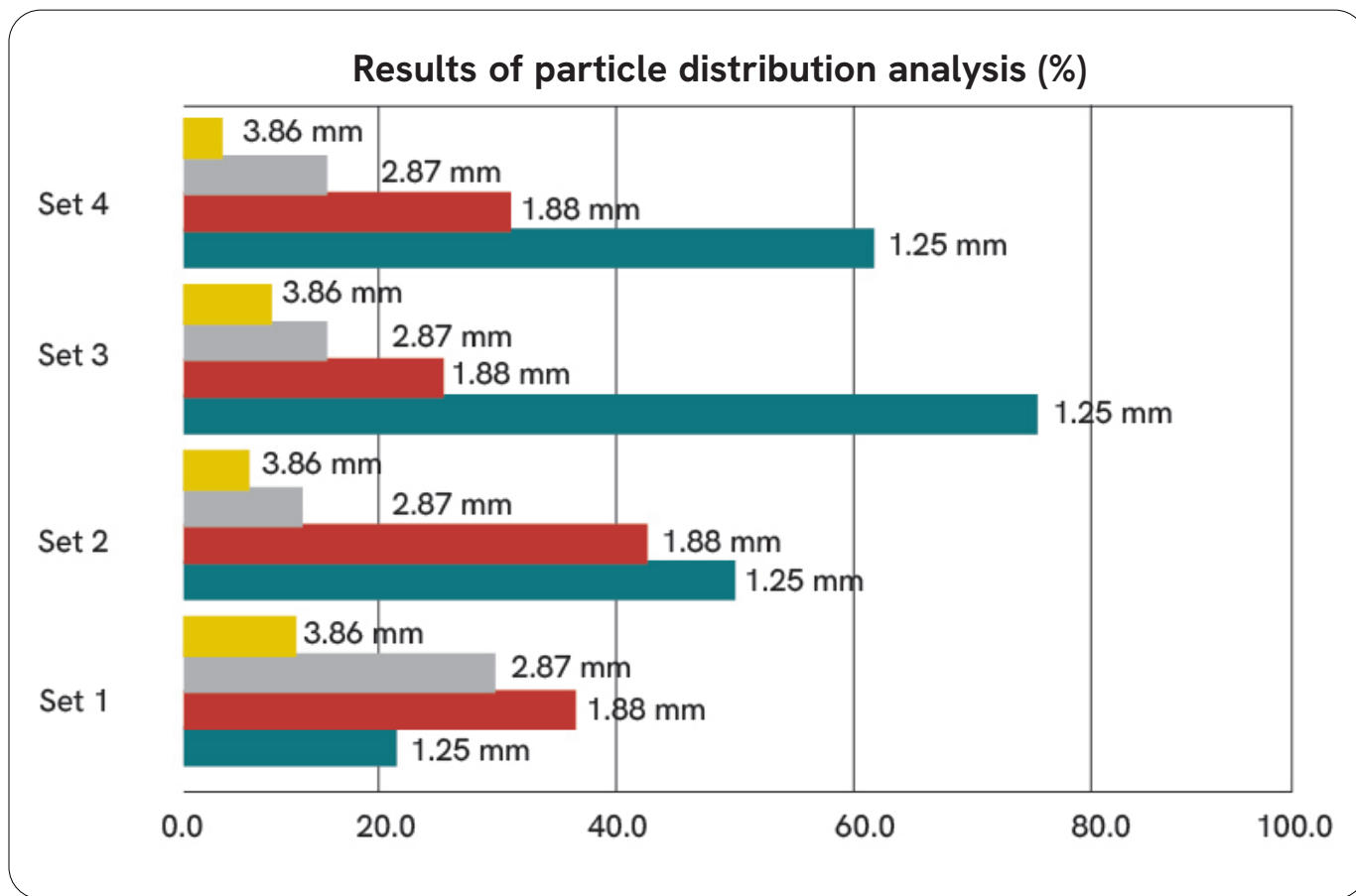### Results of particle distribution analysis (%)

Figure 2: Using the Omni Bead Ruptor 96 bead mill homogenizer, smaller particle sizes of cannabis are produced with increased cycles of homogenization. Set 1 (15 secs) shows 24 % of homogenate is ≤ 1.25 mm, while 11 % of the homogenate is ≤ 3.86 mm. Set 3 (15 secs x 4) shows the smaller particle size is over 60 % of the homogenate and remains through Set 4 (15 secs x 6). Smaller particle size allows for higher CBD extraction and accurate potency testing (Table 3), and easier mixing.

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Table 2: As homogenization cycles increase, homogenate particles become smaller and more CBD can be extracted and measured. Set - 1 (15 secs) show 5.4 % potency, however Set - 4 (15 secs x 6) shows a 15 % increase in CBD extraction and potency.

Overall results of extraction efficiency of cannabidiolic acid by homogenization parameter setting						
	Sample #	Concentration of cannabidiolic acid (CBDA) in hemp flower (%)				
		CBDA (%)	Mean value	STDEV (%)	RSD (%)	Relative extraction efficiency (%)
Set - 1	1	5.402	5.498	0.083	1.507	100.0
	2	5.546				
	3	5.545				
Set - 2	4	5.683	5.530	0.143	2.577	100.6
	5	5.401				
	6	5.506				
Set - 3	7	6.165	5.589	0.513	9.172	101.7
	8	5.421				
	9	5.182				
Set - 4	10	6.641	6.335	0.458	7.223	115.2
	11	6.555				
	12	5.809				