Mass Balance Analysis for Batch CO_2 Extraction of Hemp (Cannabis Sativa)

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This article was adapted from a presentation given at Waters Symposium on Extraction and Analytical Testing, 14th November 2017, Las Vegas, NV. The goal is to highlight briefly current CO_2-based extraction technology, and discuss considerations for adopting and implementing this technology for the cannabis industry. As an example, a processing workflow, method parameters, and routine analytical mass balance data will be presented for CO_2 extraction of Vermont Hemp using Waters Bio-Botanical Extraction System, with the goal being to describe a workflow for performing targeted extractions and monitoring batch performance.

What is Modern SFE?

Historically, supercritical fluid extraction (SFE) refers to a technique for extracting compounds of interest (COI) from solid or semi-solid substances using a supercritical fluid as the primary component of the extraction solvent [1][2]. A supercritical fluid results when a solvent is heated and pressurised above its critical temperature and pressure (Figure 1). Today however, the term SFE is also commonly used to describe the general use of carbon dioxide (CO_2) as the extracting solvent, regardless of its physical state. The flexibility to operate in significantly diverse temperature and pressure space is a major advantage of CO_2 extraction because the solvating strength of CO_2 changes significantly with temperature and pressure [1][3]. For this reason, the terms 'CO_2 extraction' and 'SFE' are often used interchangeably since extraction processes in modern instrumentation take place in both the liquid (subcritical) region and supercritical region.

CO_2 A Natural Fit for Cannabis

Carbon dioxide-based extraction is an extremely attractive alternative to traditional liquid and light hydrocarbon extractions for the bulk processing of natural products, and this is especially true for products eventually destined for human consumption [2][4]. As a result, SFE has become widely utilised by manufacturers in the emerging cannabis industry to extract, concentrate, and isolate active ingredients from the cannabis plant [5]. SFE extracts are particularly advantageous compared to liquid solvent extracts in that there is no residual solvent present in the final extract and the use of toxic or potentially dangerous solvents such as butane, hexane, and chlorinated solvents can be avoided [1][2][4]. Therefore, the time-consuming steps required to remove these unwanted solvents are lessened in the extraction workflow. SFE is a versatile technique that is able to accept the wide variability of starting materials inherent in the production of natural products [2][4]. The extracts can then be further refined, purified, analysed, or directly incorporated into final products depending on the goal of the workflow [4]. SFE acts as a hub in the processing workflow (Figure 2); it prepares the sample for multiple paths in a single step [6]. Similarly, the ability to fractionate, or create multiple extract fractions, allows manufacturers to develop multiple processing streams and products from a single CO_2 extraction.

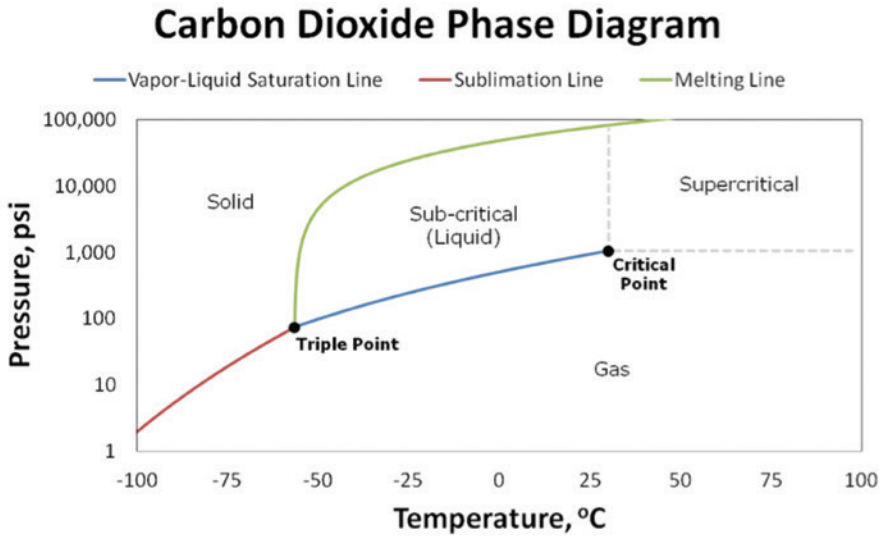

Figure 1: Phase diagram for carbon dioxide, generated using NIST RefPROP V.9.1 [3]. The three common phases (solid, liquid, gas) are shown, along with the supercritical region above the critical point. The critical temperature for CO_2 is 31°C, and the critical pressure is 1,070 psi (74 bar). Above this point, the gas and liquid densities converge into a single uniform phase with high diffusivity, low viscosity and negligible surface tension.

In the cannabis industry extraction serves as a process step that adds value to a product. For example industrial hemp (cannabis sativa) has a number of benefits associated with its production from bioremediation [7], use as building materials [8], and isolation of therapeutic constituents such as cannabinoids (primarily cannabidiol, CBD) and a variety of terpenes, flavonoids, and fatty acids [5]. Since solvent strength in CO_2 is determined by the operating

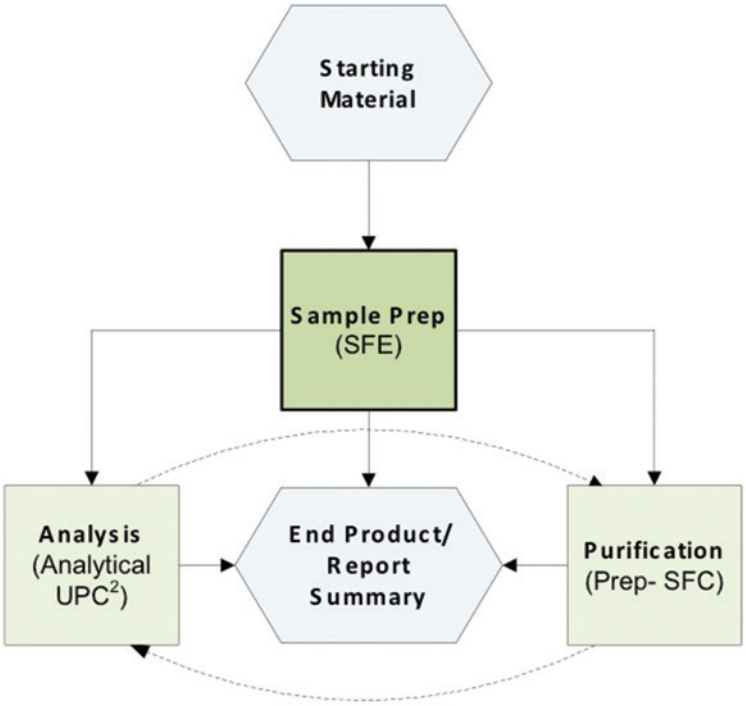

Figure 2: General processing workflow utilising SFE and other CO_2-related technologies [6].

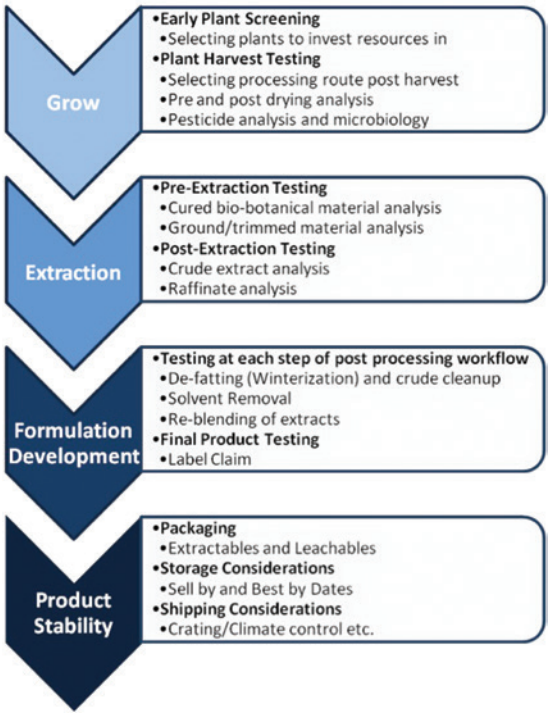

Figure 3: Important cannabis production testing checkpoints.

temperature and pressure, specific classes of compounds in the plant are targeted by carefully controlling the temperature and pressure during extraction and collection of the extract.

Extraction Analysis

Process analysis is essential during each stage of the cannabis production workflow (Figure 3). However, many cannabis production facilities contract out all of their samples to third-party analytical testing laboratories. Since each sample carries a significant price tag, processors are selective in the samples they submit for analytical testing. This lack of analytical information produces a knowledge gap with regard to basic quality control checkpoints and formulation research and development that can result in workflow inefficiencies and inconsistent products. In addition, testing results can take a week or more for labs to turn around samples, meaning acute issues linger until results are returned and corrective actions are made. Moving analysis in-house, where the samples are generated, significantly reduces turn-around time for receiving feedback about a particular process. This results in increased productivity and minimises potential quality issues by identifying them early.

One of the most important tools extraction facilities can use to track extraction quality and performance is collecting detailed mass balance data. Mass balance data is a simple 'mass in' and 'mass out' calculation essential for tracking batch extractions; the goal is to account for every gram of high value product during each processing step. During extraction this is accomplished by assaying the raw bio-botanical material for specific COIs, the target compounds from plant extraction. Since mass is conserved throughout the extraction process, the amount of COI expected to be present in the extract can be calculated and the actual yield determined post extraction. This gives a better idea of the true efficiency and return on the extraction. Following is an example of how routine mass balance analyses can be used to help monitor batch extractions of a hemp feedstock.

Routine Mass Balance Analysis for the Extraction of Hemp

Experimental:

Raw hemp extract was generated from a 20 lb feed stock of Vermont Hemp (Cannabis Sativa) using Waters 5 litre Bio-Botanical Extraction System (Figure 4), controlled by ChromScope Software v.1.6 (Waters Corporation, Milford, MA, USA). Seeds and stems were removed from the raw hemp by hand and the buds and leaves ground, homogenised, and divided into five 4 lb bags (Figure 6). Plant and extract chromatographic analyses were performed by ProVerde Laboratories, Inc (Milford, MA, USA). A sample was analysed from each bag of bio-botanical material, with the average total cannabinoid content being 5.04 wt%.

Table 1: Bio-Botanical Extraction System method parameters for CO_2 extraction.

Extraction Parameters	Method Condition	Collection Parameters	Method Condition
Flow Rate	170 g/min	CS1 Pressure	138,158 bar
Extraction Pressure	344 bar	CS1 Temperature	45 °C
Extraction Temperature	50 °C	CS2 Pressure	75 bar
Time	210 min	CS2 Temperature	40 °C
		CS3 Pressure	53 bar
		CS3 Temperature	35 °C

Table 2: Detailed extraction mass balance data for six hemp extractions. Cannabis assays were performed by ProVerde Laboratories and Mass balance calculations were performed using Waters Bio-Botanical Mass Balance Calculators at www.waters.com/massbalance

Extraction Number	Starting Mass (g)	Mass Extract CS1 (g)	Mass Extract CS2 (g)	Total Mass Yield (g)	wt% Cannabinoid Starting Material	wt% Total Cannabinoid Extract CS1	wt% Total Cannabinoid Extract CS2	wt% Acidic Cannabinoid CS1	wt% Acidic Cannabinoid CS2	Percent Cannabinoid Yield
1	1746	50.99	75.95	126.94	5.04	67.67	50.79	62.32	40.63	91.38
2	1664	28.94	94.39	123.33	5.04	59.13	50.92	55.86	43.48	87.71
3	1564.5	27.19	92.38	119.57	5.04	61.03	51.92	58.44	44.01	90.77
4	1589	20.98	95.02	116.00	5.04	60.14	52.03	57.49	43.88	85.93
5	1474	9.11	88.31	97.42	5.04	54.89	58.11	53.55	49.58	82.73
6	1383.46	6.50	80.24	86.74	5.04	53.51	55.89	52.00	48.00	80.34
Average	1570.16	23.95	87.72	111.67		59.40	53.28	56.61	44.93	86.48
Standard Dev.	129.78	16.13	7.93	15.97		5.03	3.01	3.68	3.28	4.38

Six replicate extractions were performed using Waters Bio-Botanical Extraction System (Figure 4, Figure 5). A total of 670 g raw extract (7.11 wt% Total Mass Yield) was generated from the 20 pound hemp feedstock. Three distinct extract fractions, one from each cyclone separator (CS 1-3), were collected from each extraction (Figure 6). CS 1 fraction was homogenised and analysed and CS 2 and 3 fractions were combined, homogenised, and analysed as a single fraction. Aggressive extraction conditions were utilised in this study (Table 1). Approximately 1,600 g of plant material was packed into a 5 L extraction vessel and extracted for 210 minutes at 344 bar and 55°C. An average of 86% (SD=4%) of the available cannabinoids were extracted and collected (Table 2). Extract was drained from the cyclone separators every 30 minutes and stored in a refrigerator until analysis. For each extraction, five samples were submitted for analytical testing: the hemp feedstock for that batch, extract fraction 1, extract fraction 2, ethanol rinse, and raffinate; a detailed mass balance was performed for each extraction (Table 2). Effluent CO_2 was collected and recycled; approximately 2.5 full extractions were performed per 50 lb tank of CO_2. The entire system, with the exception of the CO_2 recycler, was vented and cleaned between extractions.

Figure 4: Waters Bio-Botanical Extraction System (BBES). The pumps are on the bottom, 5 L extraction vessels to the left, and three 2 L cyclone separators at the top right.

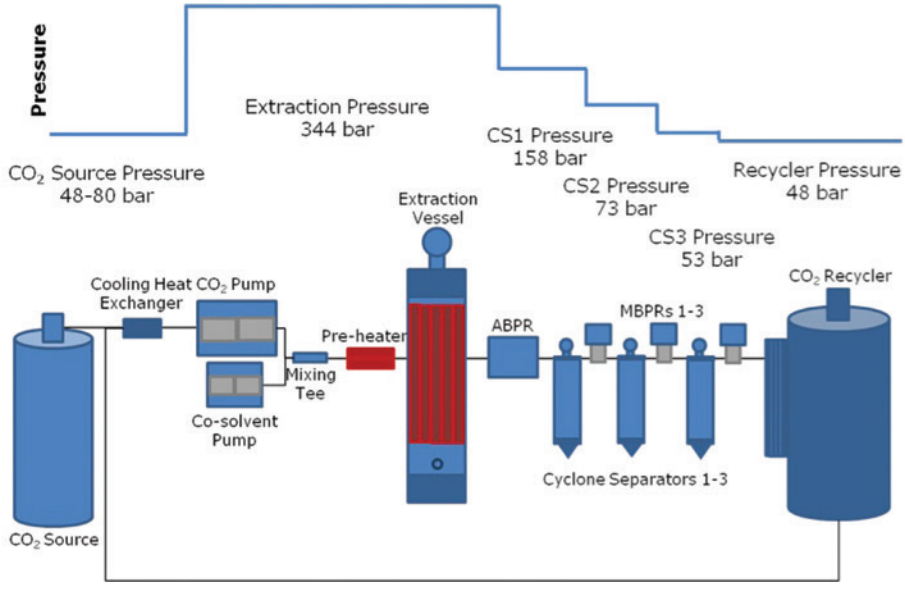

Figure 5: BBES block diagram. System flow is from the left to the right.

Discussion:

Six replicate extractions were performed over the course of 3 weeks. Significant variability in the amount of material loaded in the 5L vessel was observed, with a standard deviation of 130g of raw bio-botanical plant material (Table 2). As a result, inconsistencies in the total mass of extract collected were observed, ranging from 86.7g to 126.9g. The mass collected in each collection vessel is shown in Table 2, and Figure 7. The total cannabinoid yield under the CO_2 conditions employed was more consistent ranging from 80% to 91% (SD 4.4%) (Table 2, Figure 8). Still, there was a consistent decrease in both the amount of material loaded into the extraction vessel and the cannabinoid percent yield; this means the extraction was less efficient with less material loaded in the extraction vessel. This result is surprising because the amount of solvent used per gram of hemp is greater when less material is

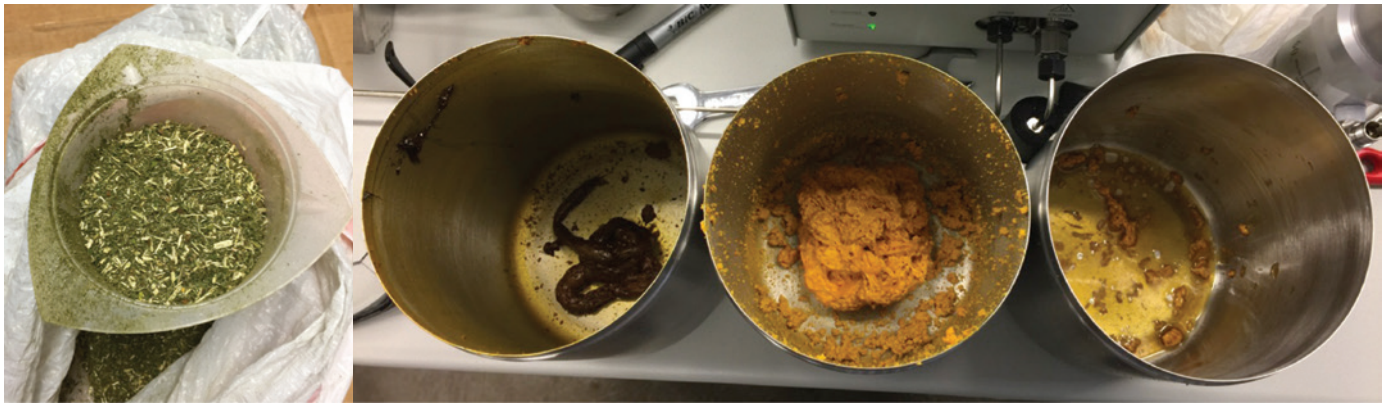

Figure 6: Vermont Hemp raw bio-botanical material (left) and extracts generated from each cyclone separator (right); from left to right CS1 2300 psi (158 bar) 45°C, CS2 1050psi (75 bar) 40°C, CS3 700 psi (53 bar) 35°C. Note: Under these conditions the dark plant pigments (CS1) and light volatile oils (CS3) are fractionated from the bulk extract (CS2).

loaded in the vessel, since the runtime and flow rate were equivalent. Temperature, pressure, run-time, and flow rate data was verified for all six runs using ChromScope software (data not shown) and no significant difference in operating parameters were observed for the 6 runs. It is possible that vessel packing heterogeneity or changes in the hemp feedstock over time resulted in the decreased extract yield. Further work should be done to investigate the effect of feedstock variability and vessel packing heterogeneity on cannabis extraction outcomes.

By extracting with CO_2 only, it was possible to effectively separate the dark plant pigments and light volatile oils from the bulk extract (Figure 6). By generating three distinct fractions from a single extraction, extraction facilities are able to generate multiple processing streams and products from a single extraction. For example, in this scenario the dark CS1 fraction, ~20% of the total extract, was directed towards a CBD-A purification pathway encompassing chlorophyll and wax removal followed by SFC purification.

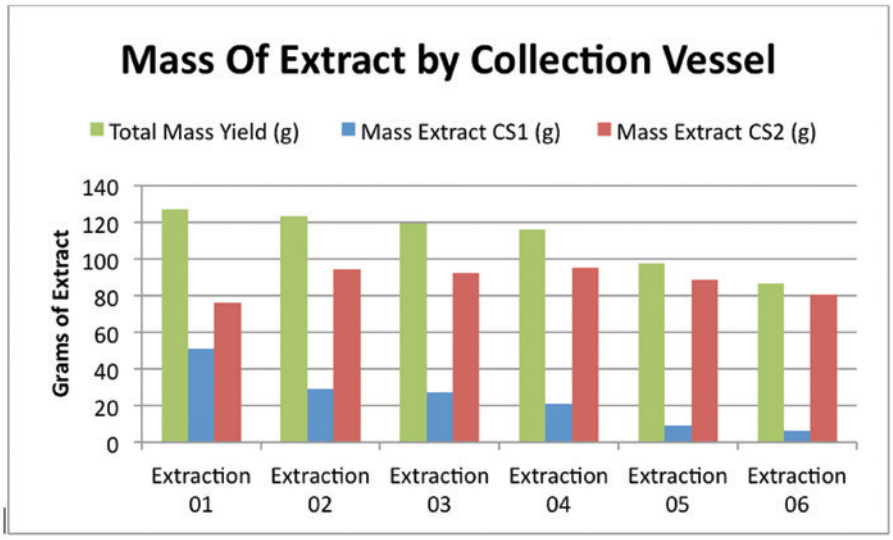

Figure 7: Collection vessel mass data for six hemp extractions. Identical conditions were run with exception to the CS1 pressure for extraction 1; CS1 pressure was 138 bar for CS1 in extraction 1 and 158 bar for the five subsequent extractions.

Fractions 2 and 3 were combined and directed towards a more traditional pathway involving only wax removal.

In the raw bio-botanical material, cannabinoids are present as the acidic precursors to the 'active' neutral cannabinoids [5]. Upon degradation by heat and light, the acidic cannabinoids convert to the neutral forms [9]. While most of the cannabinoid research has focused on the neutral forms of the cannabinoids, there has been increasing interest in therapeutic

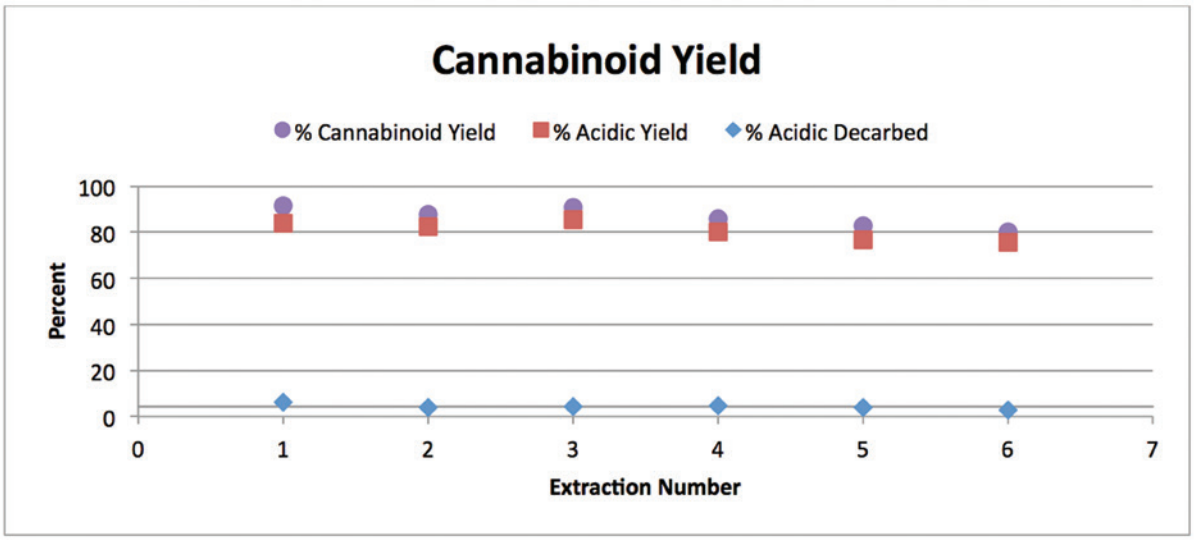

Figure 8: Cannabinoid yield and decarboxylation data for the six hemp extractions performed in this study.

properties of the acidic cannabinoids as well [10]. As such, it is becoming increasingly important to be able to preserve the natural acidic cannabinoids throughout a processing cycle. Figure 8 shows total cannabinoid and acidic cannabinoid yields for the six extractions performed in this study, along with the percent acidic cannabinoids that were decarboxylated during the extraction cycle. It is clear that even under aggressive extraction conditions, the amount of acidic cannabinoids (primarily CBDA) decarboxlyated is quite small, ~4%.

The flexibility of CO_2 extraction allowed for the generation of three distinct hemp extract fractions from a single extraction. Since there is variability inherent in working with natural products, the ability to generate multiple distinct processing streams from a single extraction is a major benefit to high pressure SFE with fractionation; this allows processors to develop specific workflows for a particular consistent desired outcome. Detailed mass balance information combined with same day in-house testing quickly identified losses in production yield due presumably to vessel packing heterogeneity. Preliminary data presented here suggests that with all other parameters the same, cannabinoid percent yield could be improved by as much as 10% by consistently packing the extraction vessel from run to run under the conditions used in this study. However, additional work needs to be done to fully understand these effects on the production process.

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