Analysis of Cannabinoids in Hemp using QuEChERS Extraction, SpinFiltr™ dSPE Cleanup and LC-MS/MS Detection

UCT Part Numbers

ECMSSC-MP
Mylar pouch containing 4g $MgSO_4$ and 1g NaCl

ECQUSF54CT
SpinFiltr™ dSPE cleanup tube 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18 and 50 mg ChloroFiltr®

SLAQ100ID21-3UM
Selectra® Aqueous C18 HPLC column 100 × 2.1 mm, 3 µm

SLAQGDC20-3UM
Selectra® Aqueous C18 guard cartridge 10 × 2.1 mm, 3 µm

SLGRDHLDR-HPOPT
Guard cartridge holder

Summary:

Thirty-nine states have defined industrial hemp as an agricultural product distinct from cannabis. Nineteen states conducted hemp research in 2017, including Colorado, Hawaii, Indiana, Kentucky, Minnesota, Montana, Nebraska, Nevada, New York, North Dakota, Oregon, Pennsylvania, Tennessee and Washington.

This application note outlines a QuEChERS method for the analysis of hemp for three cannabinoids. Sample purification is carried out using UCT's new cleanup product SpinFiltr™, which combines the convenience of classical dispersive-SPE (dSPE) with an ultrafiltration tube containing a 0.2 µm filter membrane to simultaneously remove unwanted matrix components and filter the sample prior to LC or GC analysis. The SpinFiltr™ dSPE tube uses PSA, C18 and ChloroFiltr® sorbents for sample cleanup. ChloroFiltr® is a unique polymeric sorbent designed for the removal of chlorophyll. Liquid chromatography, using a Selectra® Aqueous C18 column, coupled to tandem mass spectrometry (LC-MS/MS) is used for analysis of the cannabinoids.

SpinFiltr™

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Sample Pretreatment:

Hemp should be ground to a fine powder using cryogenic milling (e.g. SPEX 6775 Freezer/Mill®). For this work a large quantity (100 g) of hemp was thoroughly blended in a Robot-Coupe® using dry ice to generate a homogenous sample for use during method development and recovery experiments.

Figure 1. Hemp sample before (left) and after (right) homogenization with dry ice.

QuEChERS Procedure:

1. Weigh 1g of hemp sample into a 50 mL polypropylene centrifuge tube.
2. Add internal standard(s).
3. Add 10 mL ultrapure water, vortex briefly, and allow sample to hydrate for 15 min (improves extraction efficiency).
4. Add 10 mL acetonitrile containing 2% formic acid.
5. Add the contents of the **ECMSSC-MP** Mylar pouch and shake for a minimum of 5 minutes (by hand or mechanically). For this work a Spex 2010 Geno/Grinder® was used (1500 RPM).
6. Centrifuge the sample at ≥ 3000 × g for 5 minutes.
7. Transfer 1 ml of supernatant to a SpinFiltr™ dSPE cleanup tube (**ECQUSF54CT**).
8. Vortex the sample for 30 seconds.
9. Centrifuge the sample at ≥ 3000 × g for 5 minutes.
10. Transfer the purified and filtered sample extract into an autosampler vial for analysis.

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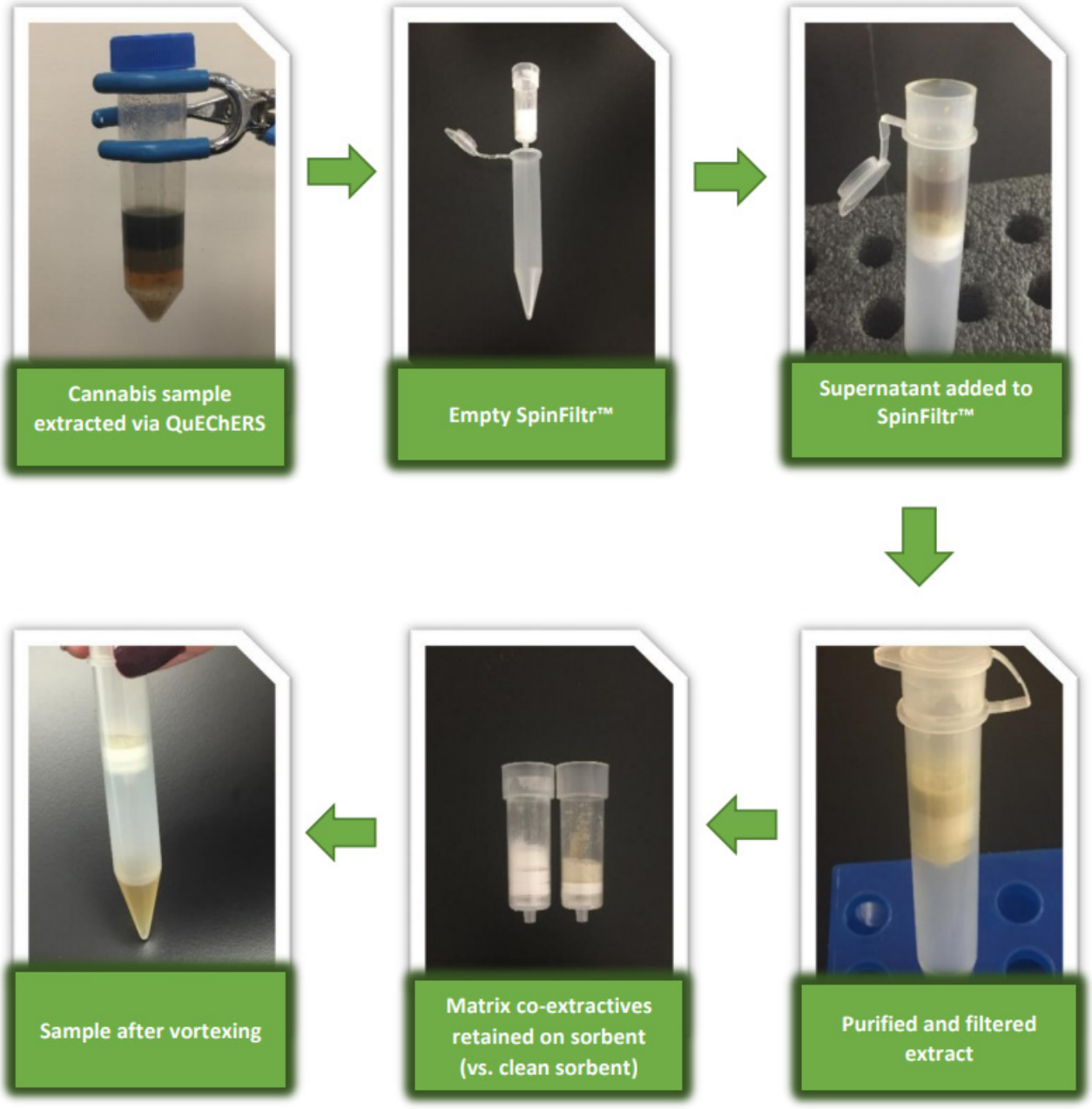

Figure 2. Schematic of the SpinFiltr™ dSPE cleanup procedure.

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LC-MS/MS Parameters:

Table 1. Instrumentation	
UHPLC system	Thermo Scientific™ Dionex™ Ultimate™ 3000
MS system	Thermo Scientific™ TSQ Vantage™ (MS/MS)
HPLC column	UCT Selectra® Aqueous C18, 100 × 2.1 mm, 3 µm (p/n: SLAQ100ID21-3UM)
Guard column	UCT Selectra® Aqueous C18, 100 × 2.1 mm, 3 µm (p/n: SLAQ100ID21-3UM)
Guard column holder	p/n: SLGRDHLDR
Column temperature	40 °C
Flow rate	300 µL/min
Injection volume	5 µL

Table 2. Mobile Phase Gradient		
Time (min)	**% Mobile Phase A (Water + 10mM NH_4HCO_2)**	**% Mobile Phase B (Methanol + 0.1% formic acid)**
0.0	40	60
0.5	40	60
3.0	5	95
7.0	5	95
7.1	40	60
10.0	40	60

Table 3. MS Parameters and Retention Times						
Analyte	**RT**	**Parent ion**	**Product 1**	**CE 1**	**Product 2**	**CE 2**
CBD	5.26	315.0	193.1	20	123.0	30
CBN	5.75	311.1	223.1	19	293.2	14
THC	5.91	315.2	193.1	19	123.1	31

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Conclusion:

The method outlined in this application note allows for the simultaneous analysis of three cannabinoids in hemp in one simple QuEChERS extraction procedure, thereby saving time, sample and cost. Sample cleanup is carried out by dSPE using UCT's new Spinfilter™ product which purifies and filters the sample in one easy step. Chlorofiltr® dSPE sorbent was used to selectively remove chlorophyll. Analysis of the samples was performed by LC-MS/MS utilizing a Selectra® Aqueous C18 HPLC column. With the increase of hemp usage across the United States, this simple method will be beneficial for any research facility wanting to ensure the cannabinoid content in their product.

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