

# Liquid-liquid extraction and ultraviolet visible spectroscopy methods for distinguishing between hemp and marijuana



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## Introduction

Passage of the Farm Bill in December 2018 legalized cannabis containing less than 0.3% tetrahydrocannabinol (THC), otherwise known as hemp (1). This creates problems for law enforcement since current presumptive test kits either 1) don't work at all or 2) work somewhat in differentiating between legal and illegal hemp crops. This problem exists because most hemp crops and hemp products contain low levels of THC and the carboxylated form, THCA. Our approach involves the advancement of an efficient, mobile, liquid-liquid extraction (LLE) that provides presumptive, qualitative forensic evidence of the chemical extract of a bud or other plant material. This research is focused on developing a kit that functions in a similar manner to NIK kits, commonly used by law enforcement, where all components of the kit are contained within a bag. The current NIK kit for Marijuana provides a false positive when Hemp is placed in the bag, thus creating the need for a more reliable test (2). The evidence would later be sent to a crime lab for definitive analysis and quantitation of THC by ultraviolet-visible spectroscopy (UV-vis). This research has focused on the utilization of liquid-liquid extraction techniques and commercially available stains. The methods presented are rapid (requiring no more than five to six minutes to complete). The differentiation between two lots of commercially available hemp and seven lots of marijuana obtained from the Cookeville City Police will be presented.

## Background

The Boles group focuses on analyzing current presumptive drug test kits and improving their effectiveness, while lowering financial, environmental, and health costs. This kit has a dual purpose which benefits the agricultural community- to provide an inexpensive kit for use by rural hemp farmers to determine the legality of their crop.

## Methods

### I. Liquid-Liquid Extraction

The standard method for the Boles kit is described below. This method can easily be adapted to a single pouch.

1. Obtain approximately 0.05 g of plant material in question and loosely pack into vial labelled "vial A."
2. Add 2 mL of proprietary organic extraction solvent to vial A.
3. Mix by shaking for 30 seconds.
4. After mixing is complete, remove 2 mL of the solvent (containing extracted cannabinoids) from vial A and add to a separate vial labelled "vial B."
5. To vial B, add 0.25 mL of 0.1M NaOH and then 0.5 mL of proprietary stain solution.
6. The aqueous layer (on top) shows a distinction between hemp and marijuana by color difference, red-orange and maroon, respectively. A maroon aqueous layer indicates the sample contains greater than 0.3% THC and is positive for marijuana.

### II. Ultraviolet-Visible Spectroscopy

This method to be performed in a forensic laboratory.

1. On the UV-vis, run a blank, quartz cuvette with 100  $\mu$ L methanol, 100  $\mu$ L stain solution, 50  $\mu$ L 0.1M NaOH, and 750  $\mu$ L RO H<sub>2</sub>O; scanning from 800-270 nm at 900 nm/min
2. Perform LLE with 0.05g plant material and 2 mL methanol, and after 30 seconds of shaking, remove the solvent and utilize a filter syringe to remove any excess plant material.
3. Add 100  $\mu$ L of the filtered extractant to a quartz cuvette along with 100 $\mu$ L stain solution, 50  $\mu$ L 0.1M NaOH, and 750  $\mu$ L RO H<sub>2</sub>O
4. Run the sample from 800-270 nm at 900 nm/min and observe the lambda max value at 520 nm for a sample of marijuana. Hemp samples give a shoulder at 400 nm, which is not present in the spectra of marijuana

## Research Questions

- ❖ What is the need for this kit?
- ❖ How is it different from pre-existing presumptive tests for marijuana?
- ❖ How will it benefit the law enforcement and agricultural communities?
- ❖ Definitive in-lab test?

### I. Liquid-Liquid Extraction

Upon extraction, hemp shows a red-orange color in the aqueous phase while marijuana shows a maroon color. Different variations of the original method have been tested to provide a more sensitive test, as well as more efficient packaging.



Figure 1

- Demonstrates the results from a standard extraction
- An LLE of hemp is shown in the left vial and marijuana is shown in the right vial
- The vials are tilted to display the aqueous color more clearly



Figure 2

- Shows extractions of hemp (left test tube) and marijuana (right test tube)
- before adding stain solution, the plant material was sat in strong acid for 5 minutes



Figure 3

- The stain solution was diluted 1:4 with water, providing for a much more distinguishable difference in aqueous layer color
- As in descriptions above, hemp is shown on the left and marijuana is shown on the right.



Figure 4

- The organic solvent mixture was replaced by a single, non-carcinogenic solvent
- One, continuous layer is formed
- Hemp is shown on the left and marijuana is shown on the right

## Conclusions & Implications

Upon further literature research, an existing product was discovered that utilizes our stain and functions similarly to our kit (3). Forward direction of our kit involves creating an "all-in-one" presumptive kit that can be used to test a variety of illicit drugs in one convenient pouch, as opposed to a systematic approach currently used in law enforcement. In addition, more modifications will be applied to the spectroscopic method to ensure accurate and consistent results. Our preliminary data is promising, though challenges must still be overcome for this to be available for use in the field. We are also planning to extend this work through collaboration with the agricultural (Hemp) community.

## Materials

Hemp (plant material) has been provided by Healing Hills Farm in Sparta, TN and the Tennessee Bureau of Investigation Crime Laboratory. Marijuana (5 different lots) has been provided by Cookeville City Police (closed cases in sealed in evidence bags). Organic chemicals used for extractant, as well as stain solution, were acquired from Fisher Scientific. Pure CBD, CBD-A, THC, and THC-A standards were acquired from Cerilliant and Cayman Chemical Company.

## Results

### II. Spectroscopic Analysis

From preliminary data, marijuana samples show a lambda max of 520 nm with no secondary peaks or shoulders. On the other hand, hemp samples have a lambda max value of 500 nm with a shoulder at around 415 nm.

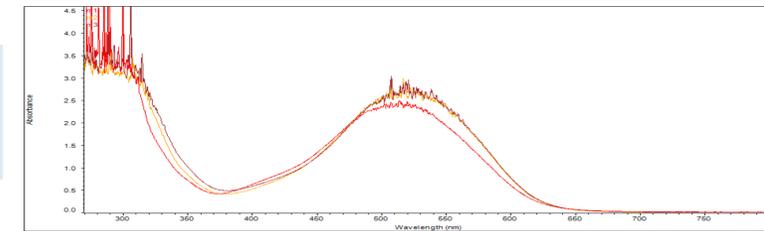


Figure 5 to the right shows three different samples of marijuana, of unknown THC concentration

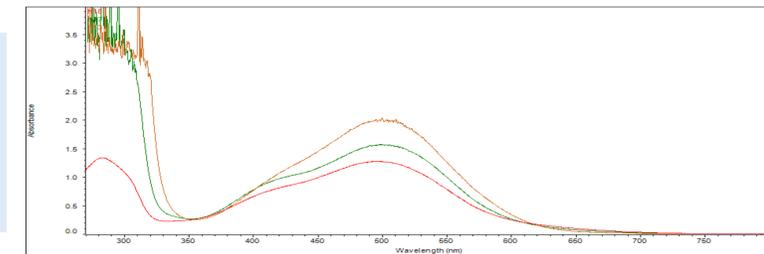


Figure 6 shows three different samples of hemp, of known THC percentages. THC concentrations are as follows: 0.066% (red), 0.146% (green), 0.280% (gold)

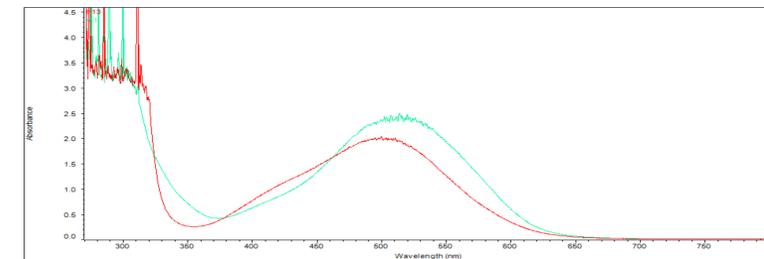


Figure 7 compares the spectra of a marijuana sample (green) vs the highest THC containing hemp sample (red)

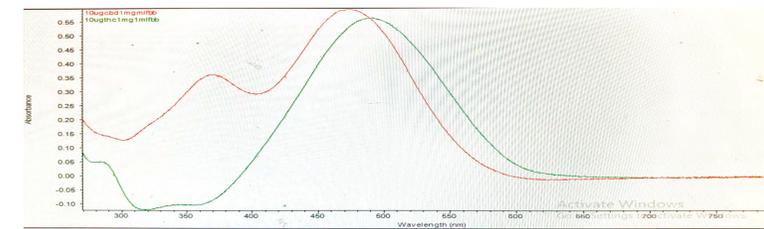


Figure 8 illustrates the spectra for THC and CBD standards. 10  $\mu$ g of each cannabinoid were scanned in the UV-vis following the same spectroscopic methods as the samples above

## References

- (1). The United States Senate Committee on Agriculture, Nutrition, and Forestry. (2018). 2018 Farm Bill (Sec. 10111 Hemp Production). Washington, D.C.: Government Printing Office.
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- (3). Symonsbergen, Kangas, Perez, Holmes. Evaluation of the NIK test: Primary general screening test for the presumptive identification of drugs. International Journal of Criminal and Forensic Science, Volume 2 Issue 5. PDF file. September 2018. <https://www.biocoreopen.org/ijcf/Evaluation-of-the-NIK-test-Primary-general-screening-test-for-the-presumptive-identification-of-drugs.pdf>

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