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 negative when Hemp is placed in the bag, thus creating the need for a more reliable kit (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.

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. 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."
4. To vial B, add 0.25 mL of 0.1M NaOH and then 0.5 mL of proprietary stain solution.
5. 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 uL methanol, 100 uL stain solution, 50 uL 0.1M NaOH, and 750 uL RO H2O; 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. The filter can then be discarded.
3. Add 100 uL of the filtered extractant to a quartz cuvette along with 100uL stain solution, 50 uL 0.1M NaOH, and 750 uL RO H2O.
4. Run the sample from 800-270 nm at 900 nm/min and observe the spectra of each cannabinoid.

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.

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 a “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 spectrophotometric 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.

References


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