Extracting and Characterizing Cannabinoids From FTA Cards: A Convenient Sampling Method for Marijuana

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Abstract

In forensic laboratories, large evidence submissions can cause the labs to lose space to store other case evidence. To combat, specifically, drug evidence storage, a method was looked at to reduce space while still maintaining a representative sample. The purpose of this research was to determine if Whatman® FTA cards would be a viable storage device to extract and detect cannabinoids. Using gas chromatography and mass spectrometry, the major cannabinoids THC, Cannabinol and Cannabidiol were detected from the cards. The cannabinoids can be transferred from the plant material to the FTA card, and once on the cards, the cannabinoids can be extracted and analyzed. As a result, the FTA cards are a viable option for storage of marijuana evidence.

Introduction

Marijuana is one of the most commonly used illicit drugs in the United States. In 2009 there were 16.7 million past month users (1). And more than 94 million Americans age 12 and older have tried marijuana at least once, according a 2003 National Survey on Drug use and Health (NSDUH) (2). With the large number of users, the chance that the law enforcement comes into contact and seizes marijuana is very high Because of the nature of marijuana seizures, retaining a representative sample of the vegetable matter that can be examined for the nature of the plant DNA and the chemical constituents, for intelligence purposes, is not a trivial problem. The marijuana can be in many different physical states ranging from fairly fresh plant material, dry, very dry and crumbly plant material, finely ground powder, hashish or even hash oil. Also, many seizures can be very large and evidence storage may be limited. To combat this issue, Whatman® FTA cards were looked at as a possible storage device for marijuana. To retain the important parts of the marijuana evidence, the material can be rubbed on FTA cards.

FTA cards have previously been used to store DNA from bloodstains, which could then be easily extracted and sequenced. They have also been previously used for marijuana DNA. Cellulose based FTA cards were shown to work for the plant material for DNA analysis by Dr. Coyle in the Forensic Science Department (3). The sampling is done by rubbing the leafy material directly on the card and can later be sampled by punching small plugs out of the stained area of the cellulose sampling area. It was then hypothesized that if biological information could be extracted from the FTA cards, then chemical information could be as well. These cards were used to see if the cannabinoids found in marijuana could be detected on the cards through chromatographic analysis. If the cards could hold both biological and chemical evidence, then they would provide more room for evidence storage.

Method and Material

A hole-puncher was used to punch holes 4.76 mm in diameter on the FTA card containing the hemp-type marijuana residue. The card was punched eight times, taking the heavily rubbed area first, for the best chance at detecting cannabinoids. Samples were then taken from the next best areas on the cards. The punches were placed in a small plastic vial sealed with a cork. Four of the punches were taken from the vial and placed into a new vial. Then 1.5 mL of Dimethyformamide (DMF) was placed in each vial. The vial was put into a beaker of water and then placed in the Branson 3510 Sonicator for 25 minutes at room temperature. The 1.5 mL of DMF was transferred to another vial without the punches. This vial was placed in the Napco Vacuum Oven Model 5831 and evaporated to dryness at about 100°C under vacuum. 50.0 μL of an internal standard solution of Phenanthrene in DMF at 0.4 mg/mL was added. The vial was turned by hand to allow the material to re-dissolve into the solution. For the HP GC-MS 5971A, 4 μL of the solution was injected. For the Agilent Technologies GC 6890N, 3 μL of the solution was injected. The GC-MS running conditions were adjusted to provide similar retention times for the major compounds on the GC.

It was noticed that during the evaporation, capillary action caused some residue to stay on the

sides of the vial where the 50 μL of DMF with internal standard could not wash it back into the solution. To fix this, the vial was filled with 0.5 mL of DMF after they were evaporated the first time. Then the vial was spun on the Mini Vortexer and evaporated again. Then 0.25 mL of DMF was added to the vial and it was spun and evaporated for a third time. The internal standard solution was then added to the evaporated vial. It is difficult to determine if these new steps have made a significant difference in the results.

The parameters for the HP GC-MS 5971A were the initial temperature was set at 207°C with no initial time hold. The ramp rate was 15°C/min to a final temperature of 270°C and was held at that temperature for 10.0 min for a total run time of 14.2 min. The GC-MS was run on split mode with a manual injection volume of 4.0 μL. The injector temperature was 250°C and the carrier gas was helium at a column head pressure of 80 psi.

The parameters for the Agilent Technologies 6890N GC-FID were the initial temperature was set at 220°C with no initial time hold. The ramp rate was 15°C/min to a final temperature of 270°C. This temperature was held for 10.0 min for a total run time of 13.33 min. The GC was run on split mode with a split ratio of 25.0:1and a split flow of 24.5 ml/min with a manual injection volume of 3 μL. The injector temperature was 250°C with a total flow rate of 28.0 mL/min. The helium flow pressure set at 9.52 psi and an average velocity of 25 cm/s. For the FID detector, the heater was set at 250°C, hydrogen flow of 40.0 mL/min, airflow of 450 mL/min, and the helium makeup flow of 45.0 mL/min.

Results

From previous work, two peaks were known to be characteristic of marijuana. There were two main peaks that were present on the chromatogram. These peaks were identified as Δ^9-Tetrahydrocannabinol (THC) and Cannabinol (CBN) which are present in drug type marijuana as seen in Figure 1.

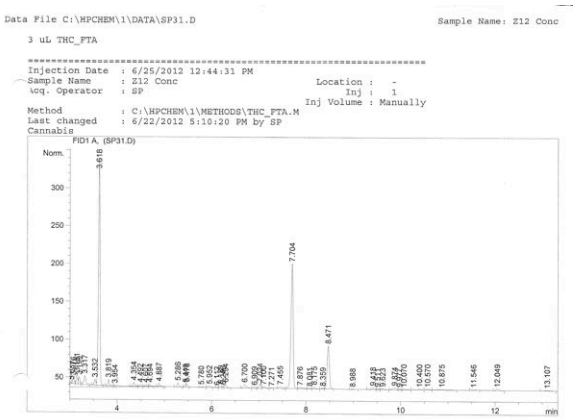

Figure 1. Gas chromatogram of drug type marijuana from FTA card.

The peak for THC is at 7.704 minutes and the peak for CBN is at 8.471 minutes.

The other cannabinoid peaks could not be identified because either the spectrum was not in the database or the quality of the spectrum did not produce a good match to compare it. The best option was to run a sample of marijuana vegetable matter to have an atlas for reference to determine if a peak was a potential cannabinoid, which can be seen in Figure 2.

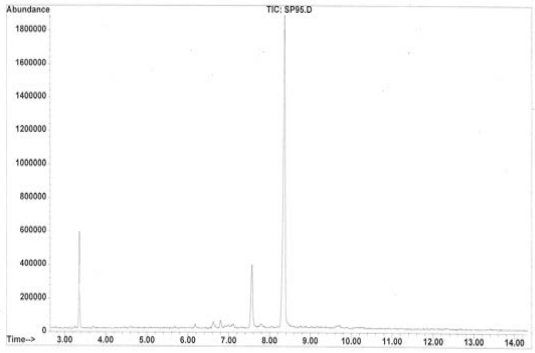

Figure 2. Gas chromatogram of drug type plant material.

Samples of hemp type marijuana were extracted to see how those chromatograms would appear. And the hemp was different in that there was little to no THC and CBN present in the chromatograms. The largest peak in the chromatograms was Cannabidiol (CBD) in Figure 3.

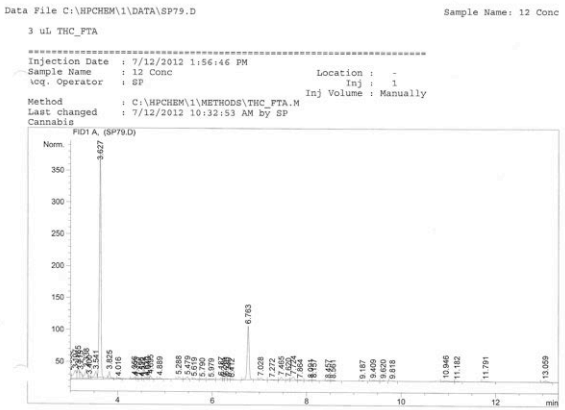

Figure 3 Gas chromatogram of hemp type marijuana from FTA card. The peak for CBD is at 6.763 minutes.

These findings also helped determine where CBD could be found on the drug type chromatographs.

To increase the sensitivity to find the other cannabinoids, two methods were performed. One method was to derivatize the extracted solution to create methyl derivatives. These derivatives would change the retention times and the fragmentation patterns of the different cannabinoids. The derivatizing agent that was used was dimethylformamide dimethyl acetal (DMF-DMA). This is because when it formed the methyl ethers the byproduct would be DMA, which is the solvent the punches are in already. So there would be no peak that could interfere with the retention times of the cannabinoids.

However, the results could not be obtained because the derivatization did not seem to work. Several options were attempted to methylate the cannabinoids. The solution that was put into the vial with the punches was the DMF-DMA to try and derivatize straight from the cards, but they did not derivatize. For a different attempt, after the first evaporation, the DMF-DMA was added to the vial to concentrated material and incubated at 30°C for 10 minutes. Neither of these options produced methyl derivative chromatograms but the derivatization process does work. A sample of the CBD standard solution was taken and derivatized using DMF-DMA.

The other method was to use the Selective Ion Monitoring (SIM) program on the Mass Spectrometer. The SIM program had higher sensitivity as it was looking for only the peaks that were inputted into the program. However it was difficult to determine from the peaks obtained which cannabinoid they matched.

Conclusion

The cannabinoids can be extracted from the FTA cards and some of them can be identified from the spectrum library. The difference between the drug type and hemp type marijuana is easily determined as well. For further research, the derivatizing method will be investigated more either by changing the agent or changing the steps for the process. The SIM program will be refined as well with different ions ion peaks to look search. A new instrument with an updated spectra library has been donated to the department, which has the opportunity to identify the other cannabinoids. There is also the possibility to determine if the FTA cards can be used for synthetic marijuana or detect illegal pesticides from samples from other countries.

References

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3. University of New Haven Honors Thesis; Ashli Ridolfi, Spring 2012.

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Biography

Sean Pickett is a junior at the University of New Haven and is a Forensic Science and Chemistry double major. He looks forward to continuing his education in graduate school with hopes of attaining his Ph.D. in Chemistry. Sean is looking to continue his research in his spare time and look for other research opportunities. Sean is currently the Vice President of the American Chemical Society Student Chapter at the University of New Haven.