TECHNICAL NOTE

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Marijuana Analysis with Recording of Botanical Features Present and Without the Environmental Pollutants of the Duquenois-Levine Test

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ABSTRACT: In order to properly document the botanical features present in a sample submitted as suspected marijuana and to reduce the problems of the disposal of the hazardous wastes produced with the use of the Duquenois-Levine Test, a protocol is described that involves recording the morphological features of *Cannabis* found in a sample and two thin-layer chromatography systems for determining the cannabinoids present. This protocol provides more information on a sample than was obtained with other, previous protocols involving the Duquenois-Levine Test.

KEYWORDS: criminalistics, marijuana analysis, Duquenois-Levine Test, TLC of cannabinoids

Much of the analytical work performed for the identification of marijuana, *Cannabis sativa* L., has been based on the articles of Nakamura [*1*] and Thornton and Nakamura [*2*]. These reports are excellent, thorough, compilations of data for the use of the microscopic identification of trichomes in the identification of fragments of *Cannabis*. The latter article, in addition to reporting on the authors' study of trichomes, delves deeply into the significance of the Duquenois-Levine Test in confirming the identification of *Cannabis*.

Since these reports were written, however, this laboratory has noted an increase in the numbers of submissions of marijuana samples that have flowers and relatively intact leaves rather than the fragments of leaflets that were common 15 years ago. Several years ago, it was realized by many of our analysts that documentation of the morphological features observed in marijuana samples was very advantageous in training analysts and was far superior to the "Micro +" documentation once used frequently. With detailed documentation, one can report on the witness stand exactly what was seen rather than being unsure whether a plethora of *Cannabis* features were found or just a *Cannabis* leaflet fragment with minimal features was present. Only recording the presence of the particular types of characteristic trichomes on leaflet fragments ignores a large amount

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of data on the sample that is extremely useful in identification. Because what is being identified is a plant or plant parts, the morphological examination of the sample with a stereoscope, therefore, is the best available mechanism for such an identification, and the documentation of the findings is imperative.

In addition, compliance with the new regulations on the disposal of chemical wastes has been added to the duties of the forensic scientist since these reports were written. Waste from performing the Duquenois-Levine Test [*2,3*] involves a complex mixture of chloroform, concentrated hydrochloric acid, ethanol, and reaction products involving cannabinoids, vanillin, acetaldehyde, and miscellaneous other chemicals. Reduction of the chlorinated hydrocarbon wastes is a primary environmental concern [*4*]; therefore, analysis protocols should develop in the direction of eliminating the Duquenois-Levine Test.

In addition to the problem of waste disposal, the Duquenois-Levine Test cannot distinguish individual cannabinoids or other compounds with similar functional groups. One of these other compounds, olivetol, is used by me to treat leaves of various plants for use as training samples. By dissolving olivetol in petroleum ether and pouring this solution on plant material other than marijuana and allowing the petroleum ether to evaporate, the sample can then be extracted with petroleum ether and produce the same result as marijuana with the Duquenois-Levine Test. Such a sample will also produce the same result as marijuana when the test is run on the unextracted plant sample directly. It would be preferable to use tests that distinguish among the cannabinoids and compounds reacting similarly to them.

This article describes a protocol for marijuana analysis that involves detailed notation of features found with stereoscopic examination of the plant material and two thin-layer chromatography systems to examine the cannabinoids present. While there is chemical waste with these TLC systems, the wastes are all clean burning solvents; their disposal is, therefore, less expensive economically and environmentally.

Materials and Methods

Microscopic Feature Description

To achieve the goal of recording the botanical features present in a submission of marijuana, the sample is visually examined, and as much as possible is viewed stereoscopically with magnifications of 10 to 40 times. The features observed are then recorded either in a number code[2] or in an abbreviated written form depending upon the preference of the examiner. Many of these botanical features have been mentioned in the U.S. Treasury Department's Marijuana, Its Identification, of 1948 [*5*] and Schultes and Hofmann's book of 1973 [*6*]; however, in Table 1 is the more detailed listing of features that I use. To give an example, the description of a marijuana leaflet on a palmate petiole would be described in my abbreviations as "Cnb 11 on palm pet," which means, "Cannabis leaflet on a palmate petiole." This one short statement documents the presence of a leaflet with cystolithic trichomes on one side, unicellular trichomes on the other side, leaflet serrations, the pinnate and marginal pattern of the veins, and the attachment of this leaflet to a palmate petiole. A notation of whether the achenes are reticulate or marbled in appearance is also of aid in any multiple species argument the defense might bring up, since the very common reticulated appearance of the achenes is indicative of the narrow definition of *Cannabis sativa* L. in Professor Schultes' key [*7–9*].

[2]Harris, J. R., Kentucky State Police Southeastern Regional Laboratory, personal communication, 1983.

TABLE 1—*Botanical characteristics that are recorded by abbreviations.*

Cannabis leaflet fragment (includes *Cannabis*-like cystolithic trichomes and unicellular trichomes on opposite sides of the leaflet particle that, itself, has a general *Cannabis* appearance)
Cannabis leaflet fragment with various features individually listed as follows: serrations, pinnate venation (that is, the pattern of the leaflet veins branching individually off of the midrib or middle vein in a featherlike pattern), marginal venation (that is, veins running along the serrated edges but vanishing before reaching the very tip)
Cannabis leaflet (the whole leaflet, or one with all the features mentioned for a leaflet fragment)
Cannabis leaf (at least one leaflet on an obvious palmate petiole)
palmate petiole
staminate flowers or parts (anthers, sepals)
pistillate flowering tops or an isolated pistillate flower
enveloping bract of the pistallate flower
stigmatic styles of the pistillate flower (when broken off from the rest of the flower)
glandular trichome
glandular trichome with head
hull (husk)
achene with reticulate or marbled pattern; the achene, which actually is the fruit of the marijuana plant, is commonly, but incorrectly, referred to as the seed
Cannabis seedling, with cotyledons, at the first, second, or third leaf stage
Cannabis stalks or stems
foreign plant material (including approximate percentage or amount)
roots
Aspergillus fruiting bodies
fungal mycelia
soil

Chemical Features Determined

Two different chemical tests in addition to the stereoscopic examination are required for reporting the identification of marijuana in the Kentucky State Police Forensic Laboratories System. For one test, it uses the hexane:ethyl ether (4:1) solvent system [*10*] with E. Merck 0.25-mm Kieselgel 60 F_{256} plates, or the equivalent, for separation and characterization of the cannabinoids. Traditionally, the Duquenois-Levine Test has been used as the other chemical test. Replacement of the Duquenois-Levine Test with a second TLC with the hexane:acetone (4:1) solvent system occurred at the Northern Regional Laboratory after five years of dual testing with these two TLC systems and the Duquenois-Levine Test. This latter system was an inadvertent modification of one used at another laboratory.[3] This modification was found to have no significant effect upon separation but it did increase the absolute R_f values by approximately 20%.

Method Testing

Various spices, condiments, and members of the botanical order, Urticales, to which *Cannabis* belongs, were examined by stereoscope at magnifications of 10 and 40 times and extracted with petroleum ether for TLC analysis. Various chemicals, including many reported to give positives with the Duquenois-Levine Test or used in the synthesis of THC [*2,3,10*] were also tested. Table 2 contains a list of the material tested. Samples were spotted about 14 mm from the bottom of a 10-cm long TLC plate; the solvent was allowed to rise up the entire plate before being removed. The plates we e then sprayed with Fast Blue 2B in a 1:1 mixture of methyl alcohol and water for visualization of the chromatographic zones.

[3]Skowronski, G. T., U.S. Drug Enforcement Administration North Central Laboratory, personal communication on a TLC system with six parts hexane to one part acetone.

TABLE 2—*A list of spices, condiments, and plants examined for features of marijuana and extracted for TLC.*

Allspice	Ginger, ground	Onion, minced and flakes
Bay leaves	Guaiazulene	Orcinol
Black pepper corns	Hackberry (*Celtis occidentalis*), leaves	Oregano leaves
Black pepper, ground	Hops (*Humulus lupulus*), leaves	Parsley flakes
Catnip	Jimson weed (*Datura stramonium*), seeds	Patchouli oil
Cinnamon, ground	Mace, ground	Red peppers, crushed
Cinnamon, stick	Metamucil	Red peppers, ground cayenne
Citral	4-methylresorcinol	Sage leaves
Cloves, ground	Morning glories seed	Sage, rubbed
Cream of tartar	Mulberry, red (*Morus rubra*), leaves	Savory, ground
Cumin seed	Mustard seed	Summer savory
Curry powder	Nutmeg	Thymol
Elm (*Ulmus americana*) leaves	Osage orange (*Maclura pomifera*), leaves	Thymolphthalein
Eugenol	Olivetol	Tobacco
Garlic powder		Tumeric, ground
Garlic salt		
Ginseng root		
Glutamate, monosodium		

Results and Discussion

Microscopic examination of the listed samples produced no sample with which an experienced examiner should have trouble. In Table 3 are listed the color and relative R_f values, normalized to delta-9-tetrahydrocannabinol (delta-9-THC), of colored chromatographic zones of the specimens and chemicals from Table 2 that produced such zones. No plant product nor other chemical was found to produce a colored chromatographic zone that could be confused with THC. The facts that delta-9-THC and cannabidiol switch relative positions as do cannabichromene and cannabinol when run on these two systems are of significance. Also, while the hexane:ether system cannot adequately separate cannabichromene from cannabigerol, it can separate cannabidiol well. The reverse occurs with the hexane:acetone system but cannabidiol and cannabichromene frequently overlap some as do cannabinol and cannabichromene. The sum of these facts allows a greater confidence in the determination of the major cannabinoids present. In addition, I have not found any mechanism to make a test sample that is not marijuana produce a confusing TLC result for either system without actually using a cannabinoid, as can be done with the Duquenois-Levine Test. It should be noted that hashish samples and ground, compressed marijuana samples are examined by instrumental means in addition to stereoscopic and TLC analysis because of the lack of many of the key botanical features.

Conclusion

Adequate stereoscopic examination with thorough documentation of morphological features present and a more detailed determination and recording of the cannabinoids present in a sample with the two TLC systems discussed provide an unambiguous identification of *Cannabis sativa* L. This combination of methods is both rapid and inexpensive and involves less toxic materials that are easier to dispose of in an environmentally safe way than testing with the Duquenois-Levine Test. Because the problem is the identification of plant parts present and not a chemical, the method emphasizes documentation of these plant parts and does not tie up instrumentation designed to identify chemicals.

TABLE 3—*Relative R_fs of various cannabinoids, spices, condiments, and chemicals that produced colored chromatographic zones with Fast Blue 2B on the two TLC systems used.*

Compound or Spice	Hexane:Ethyl Ether (4:1) TLC System		Hexane:Acetone (4:1) TLC System	
	Color with Fast Blue 2B	Relative Rf	Color with Fast Blue 2B	Relative Rf
Delta-9-THC	red	1.00	red	1.00
Delta-8-THC	red	1.10	red	1.08
Cannabichromene	purple	0.80	purple	0.89
Cannabidiol	orange	1.14	orange	0.93
Cannabigerol	orange	0.78	orange	0.72
Cannabinol	purple	0.90	purple	0.86
Allspice	yellow	0.67	yellow	0.77
Black pepper	yellow	0.0	yellow	0.27
	pink	0.63	purple	0.90
			purple	1.48
Cloves	yellow	0.86	yellow	0.87
	pink	1.08	purple	1.04
			yellow	1.54
Curry	yellow	0.0	yellow	0.08
	yellow	0.92	yellow	1.00
Eugenol	yellow	0.78	yellow	0.89
Ginger	orange	0.27	orange	0.53
	yellow	0.40	yellow	0.63
			yellow	0.74
Guaiazulene	purple	1.66	purple	1.65
Mace	red-purple	0.0	purple	0.0
	purple	0.44	purple	0.09
	purple	0.75	purple	0.30
			purple	0.70
4-methylresorcinol	red-brown	0.11	red-brown	0.34
Nutmeg	purple	0.0	purple	0.09
	purple	0.17	purple	0.16
	purple	0.25	purple	0.63
	purple	0.35	purple	0.81
Olivetol	red-brown	0.07	red-brown	0.28
	red	0.48	red purple	0.72
Orcinol	red-brown	0.05	red-brown	0.21
Red pepper	yellow	0.0	yellow	0.0
	yellow	0.25	yellow	1.05
	tan	0.10	yellow	2.00
	purple	0.73	pink	1.28
	yellow	1.73	pink	1.77
Sage	grey-green	0.10	green	0.58
			green	0.75
Savory	grey-green	0.10	green	0.50
Thymol	yellow	0.95	yellow	0.96
Tumeric	yellow	0.0	yellow	0.04
	yellow	0.96	yellow	0.75
			yellow	1.06

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