MICROGRAM

BUREAU OF NARCOTICS AND DANGEROUS DRUGS / U.S. DEPARTMENT OF JUSTICE

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Psilocybin Analysis - Reference

Doctor Wilcox, Chief, Bureau of Laboratories, and Dr. Howenstine, Division of Crime Detection, Michigan Department of Health, submitted an interesting article on the identification and analysis of the Psilocybe mushroom.

See Heim, R. et al, "Botanical and Chemical Characterization of Forensic Mushroom Specimen of the Genus Psilocybe," Journal of the Forensic Science Society, pp. 192-201, Volume 6, No. 4 (1966).

Nitrous Oxide Abuse

California Medicine, June, 1967, has an interesting exchange of letters between a pastor and a physician regarding the abuse of Nitrous oxide.

The article reports one young man sniffing the contents of a 6 lb. cylinder each week, and the man reportedly had had nine auto accidents while under the influence.

The article implied most of these cylinders were being stolen.

Ballistics Examination of Drugs

Ballistics examination of tablets and micro-analytical examination of tablets and capsules can often reveal that:

(1) The drug is a counterfeit
(2) The drug is illicit, and was manufactured on a punch common to other similar preparations being found.
(3) The drug is a product of a known, legitimate manufacturer. This, in turn, gives information on the kinds of products of various manufacturers found on the street; the extent; the geographical area; and other information. Results of examination in these cases are dependent on an authentic sample of the manufacturer being in our drug library. These authentics, which include formulation data, are collected by our agents in the course of accountability investigations.

Ballistics Examination of Drugs (cont.)

If you would like to have a ballistics examination made on any tablets, send them in. See MICROGRAM No. 5 for instructions on how to submit evidence.

For our intelligence purposes, we are particularly interested in seeing LSD, STP, and amphetamine tablets.

COUNTERFEITS

We recently examined well-made counterfeit OBEDRIN - LA Tablets (Massengill), which contained only caffeine as the active ingredient. Legally manufactured tablets contain methamphetamine, pentobarbital, and vitamins, and are used in weight control programs. OBEDRIN - LA is a double layer tablet, and is shown in the "Product Identification" section of the Physicians' Desk Reference.

DIPROPYLTRYPTAMINE (DPT)

We recently encountered DPT on parsley cigarettes. Reportedly, the research chemist manufacturer bragged that he could keep adding a carbon and stay ahead of the law.

From our pharmacologists, we learned that DPT has been found to be active by intravenous injection in a research study using humans. We do not know whether or not the compound is active when smoked. Apparently there will be no DBT, because the butyl analog has not been found to be active.

PAM

Teenagers are reportedly sniffing this aerosol product used to coat cooking utensils. FDA reports two deaths in which abuse of this product is suspected. Composition unknown at present. Propellants probably dichlorodifluoromethane and trichloromonofluoromethane.

"LBJ"

Reportedly a "new" drug, being found in gelatin capsules containing a white powder.

The underground press reports that 2000 samples have been released in New York, after a test run in Boston. Press reports "LBJ" to be a "cross" between mescaline, belladonna and "acid". With a reported 5 to 15 milligram dose.

Analysis of one capsule in the field indicates a small amount of amphetamine with other substance(s). A capsule is being analyzed in Washington, D.C.

STP

Appearing mainly on the West Coast at present.

Yellow Tablets: Round, flat, beveled, compressed, uncoated, unscored. Diameter approximately 4.9 mm; thickness about 3.3 mm; weight approximately 75 to 80 milligrams; potency range 9.1 to 10.2 milligrams STP.

White Tablets: Round, flat, beveled, compressed, uncoated, unscored. Diameter about 4.8 mm; thickness about 3.1 mm; weight about 75 milligrams; potency about 9.2 milligrams.

Capsules: Clear gelatin, containing light green powder. Length 14.5 mm; diameter 5.5 mm. (apparently a No. 3 capsule); gross weight 0.2259 grams; net weight 0.1833 grams; potency 3.2 milligrams STP.

Blue LSD Tablets

Appearing in several parts of the country. The tablets examined have been made on the same single punch press. This press was turning out LSD tablets several months ago.

Tablets now on scene are blue, round, unscored, uncoated, flat both sides, and not beveled. Diameter approximately 6.4 millimeters; thickness approximately 2.3 millimeters; weight about 103 milligrams; contain about 136 micrograms of LSD.

The tablets purchased several months ago, were purple, blue, green, or white. Tablet weights at that time ranged from 84.7 to 99.7 milligrams, with a mean weight of 94.7 milligrams.

A large amount of dextrose monohydrate has been found in all but one sample. Lactose Monohydrate was found in the exception. Most tablets, in the earlier analyses, contained small or trace amounts of ascorbic acid, corn starch and/or calcium salts. Recent tablets have shown a larger amount of corn starch.

MICROCRYSTAL AND COLOR TEST FOR STP (DOM)

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The following four tests have been found to be very useful as a rapid laboratory screening procedure for STP ("DOM", 4-methyl 2,5-dimethoxy-amphetamine). The first two are color tests, and the other two are microcrystal tests. The color tests do not seem to be affected by the presence of LSD, but there is indication that LSD does interfere with the microcrystal tests. All tests are of the direct type, and are performed simply by adding a drop of reagent solution to a little powdered tablet material or capsule material (or liquid) and observing the reaction microscopically.

The color tests are best performed on a white spot-plate, or glass slide with white background, and the color(s) developed should be observed with a wide-field microscope at 10X.

The microcrystal tests should be done on a glass slide, and observed at 78-125X with a polarizing microscope, preferably, both with and without the analyzer inserted. A regular compound microscope may also be used, although many diagnostic anisotropic properties of the crystals are thus lost. Always run side-by-side tests, comparing with authentic STP.

Reagents*:

1. Mecke's Reagent: 1 g. selenous acid in 200 ml. conc. H_2SO_4.
2. Fröhde's Reagent: 1 g. ammonium molybdate in 10 ml. conc. H_2SO_4. Keep tightly closed. Oxidizes easily and turns blue.
3. Bismuth Iodide in dil. H_2SO_4: Dissolve 1.25 g. KI in 2.0 ml H_2O, and add 2.5 ml. H_2SO_4 (1+3) and 0.5 ml. stock conc. Bi $(NO3)3$ solution (prepared by dissolving 50 g. bismuth subnitrate in 70 ml. HNO_3 (1+1) and diluting to 100 ml. with H_2O). Prepare fresh when the most characteristic crystals fail to form or form too slowly (perhaps every several weeks).
4. Saturated aqueous Picric Acid.

Tests:

1. Mecke - Greenish yellow color with reddish orange (rust-colored)ppt.
2. Fröhde - Yellowish green, then green, then blue.
3. Bismuth Iodide - First orange-reddish droplets immiscile in reagent

form. Then gradually crystals form. The most characteristic are yellow serrated blades, often in form of asymmetric X or bow-shaped. Rosettes of closely packed thin needles (shaggy appearance) also may form.

4. Picric Acid: The most characteristic crystals, which form gradually, are asymmetric starlike clusters of highly birefringent platy pale yellow crystals. You may also get highly birefringent elongated orange-yellow jointed or jagged plates and rosettes of yellow blades and needles with gray to orange-yellow interference colors.

*For reagents (1) & (2) see Bamford, Frank, Poisons, Their Isolation and Identification, 3rd edit., p. 248. For reagent (3) see A.O.A.C. 10th edit., 32.279(e), except that we have found that the reagent need not be prepared fresh daily as directed.

THE IDENTIFICATION AND QUANTITATIVE DETERMINATION OF PSILOCYBIN IN TABLETS

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Psilocybin [indol-4-ol,3-(2-(dimethylamine) ethyl)], a hallucinogenic drug occurring naturally in the mushroom Pscilocyba mexicana (1), has recently appeared on the illicit drug market. Pharmacologically, psilocybin and other LSD-type drugs both stimulate and depress the central nervous system, impairing coordination, judgment, and sense of time; the degree of reaction and duration of effects depend on the dosage used. Psilocybin is about 1/100 as potent as LSD and one tablet dose usually contains about 4-8 mg (2).

Because of FDA's responsibilities under the Drug Abuse Control Act, a method is needed for identifying psilocybin and determining it quantitatively in unknown samples. Such a method has been developed and is given in detail below.

In this procedure, psilocybin is extracted into methanol and the ultraviolet (UV) absorbance of the solution is determined at 268 mμ; it is then compared with the characteristic UV spectrum of a standard psilocybin solution which has an absorptivity of 220 at 268 mμ. Psilocybin is further identified by thin layer chromatography (TLC).

Method

Reagents and Apparatus

(a) Celite 545.-Acid-washed (3).

(b) Methanol.-Anhydrous, reagent grade.

(c) Mobile solvent.-Mix 50 ml n-propanol (reagent grade) and 35 ml 5% ammonium hydroxide.

(d) Chromogenic spray (Ehrlich's reagent).-5% solution of dimethylamine-benzaldehyde (Eastman 95) in concentrated HCl.

(e) Psilocybin reference standard.-0.5 mg psilocybin (Sandoz Pharmaceutical, Hanover, N.J.) per 100.0 ml methanol (b).

(f) Column.-Partition chromatography glass column ca 25 mm in diameter x 200 mm long.

(g) TLC plates.-Put a 250 μ layer of silica gel G on plates 8 x 8". Dry for 30 min. at 80°C. Store in desiccator.

Procedure

Assay.-To a 100 ml beaker add powdered sample equivalent to 0.5 mg psilocybin, 1 g Celite, and 1 ml methanol, and mix thoroughly. Transfer mixture to partition column containing a layer of a mixture of 3 g Celite and 2 ml methanol. Dry wash the beaker and transfer to column. Wipe beaker with pledget of glass wool and place on top of column. Pack entire column firmly, place a 100 ml volumetric flask under it, and elute with 95 ml methanol. Prepare a blank column (minus sample) as above and carry through the same procedure. Dilute to volume with methanol. Determine the UV spectrum from 330 to 230 mμ in a 5 cm silica cell vs. a methanol reference. Compare the spectrum with the standard solution spectrum at 268 mμ by the base line technique and correct for blank. Calculate the amount of psilocybin in the sample. If the eluate is cloudy, the column has not been packed tightly enough. To remove this cloudiness, centrifuge at 2300 rpm for 10 min.

Identification by TLC (1).-Spot from 2 to 4 μg psilocybin (sample and standard) on a thin layer plate. Aliquots of sample and standard solution can be concentrated by air evaporation only. Develop the plate for 2 hr with the mobile solvent, remove plate from developing tank, and allow to dry. View under UV light. The psilocybin spots are faintly visible. Spray plate with chromogenic reagent. Psilocybin yields blue-violet spots with an R_f value of about 0.38.

Discussion and Results

In the above method, psilocybin was identified first by its characteristic absorbance of UV light and second by TLC. p-Dimethylaminobenzaldehyde (T.S.) was tried as the chromogenic reagent for psilocybin but the resulting spots were a very pale yellow and difficult to see. Ehrlich's reagent was therefore used. (LSD had an R_f of 1.0 when chromatographed as above.)

The following table summarizes the psylocybin recovery data for simulated tablet mixes, column recoveries and commercially prepared tablets.

Table 1. Psilocybin (PSC) recovery data

Analyst	Sample Composition	Recovered
	Simulated Mixes	
A	0.5 mg PSC/300 mg starch	101%, 102%
B	0.5 mg PSC/300 mg starch: talc (5:1)	96%, 98%
	Column Recoveries	
A	0.42 mg PSC added	99%, 103%
	Commercial Tablets (single tablet assay)[a]	
A	0.5 mg PSC declared	0.5 mg
C	0.5 mg PSC declared	0.45 mg

[a] TLC identification was positive for psilocybin.

Acknowledgment

The authors wish to thank Donald Dechert for his assistance and collaboration.

References

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(2) Medicinal Chemistry, A Series of Monographs: DeStevens, G. (Ed.), Diuretics: Chemistry and Pharmacology, Vol. I, 1963; Maxwell, G. (Ed.), Psychopharmacological Agents, Vol. IV, 1964, pp. 556-574, Academic Press, New York and London.

(3) United States Pharmacopeia, 17th Rev., Mack Publishing Co., Easton, Pa., 1965, p. 192.