

TECHNICAL NOTE

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Microchemical Identification of Gamma-Hydroxybutyrate (GHB)*

REFERENCE: Andera KM, Evans HK, Wojcik CM. Microchemical identification of gamma-hydroxybutyrate (GHB). *J Forensic Sci* 2000;45(3):665–668.

ABSTRACT: A new microcrystal test for the detection of gamma-hydroxybutyrate (GHB) is described. The silver/copper reagent consists of an aqueous solution of 0.1 g of cupric nitrate and 0.1 g of silver nitrate in 10.0 mL water. While some crystals form upon evaporation of the reagent, the test forms distinctive crystals for GHB and does not form crystals with some commonly encountered controlled substances. The reagent was also tested against some controlled substances that have similar biological activity to GHB, including flunitrazepam, and some barbiturates. No crystals were observed with these compounds. A blind test was performed to determine if GHB could be discriminated from the other compounds. Two of ten unknowns were correctly identified as GHB—one solid, one liquid. One GHB sample was not identified as GHB and the remaining seven non-GHB samples were not identified as GHB. The reagent is therefore selective for GHB, but not extremely sensitive.

KEYWORDS: forensic science, sodium oxybate (gamma-hydroxybutyrate), microcrystal test

Gamma-hydroxybutyrate (GHB) (1) has recently become a Schedule II controlled substance in California (2), is controlled in several other states (3), but is not federally scheduled. A rapid, simple test was desired for the substance. Ideally the test would have greater specificity than the chromic acid (4) or Janovsky color tests (5–7) with less preparation than needed for infrared spectroscopy (IR) or gas chromatography/mass spectrometry (GC/MS) (8). Microcrystal tests have proven themselves to be effective (9), uncomplicated tests which can be run directly on the sample (10). None were found in the usual chemical microscopy literature (11–13).

Methods

In searching for a color test for GHB, one of the authors (Evans) found a reference for a microcrystal test for butyric acid using

cupric nitrate (6). The test described a flaky green precipitate and flat hexagonal crystals. As cupric nitrate was not immediately available in the laboratory, an aqueous cupric nitrate reagent was prepared from cupric chloride (CuCl_2) and silver nitrate (AgNO_3), precipitating silver chloride (AgCl). The reagent, when added to a concentrated solution of GHB by Chamot's Method 1 (14), formed large rectangular birefringent crystals when observed for approximately 5 min under polarized light at a nominal magnification of approximately $\times 100$. Crystal growth occurred almost exclusively at the periphery of the exposed drop. Subsequent tests of reagent-sample combinations followed this regimen.

After a supply of cupric nitrate ($\text{Cu}(\text{NO}_3)_2$) was purchased, a 1% w:v reagent was prepared. No crystal growth was observed with this reagent and GHB. It was noted that in the original solution there was almost certainly some remaining silver ions (Ag^+) after the precipitation of the silver chloride. An earlier experiment had shown no crystal growth with a reagent of silver nitrate alone, so an equal weight of silver nitrate was added to the cupric nitrate solution. The new reagent, silver nitrate + copper nitrate, formed the same rectangular crystals (Fig. 1) as the ad hoc cupric nitrate reagent. Both the cupric (Cu^{++}) and silver (Ag^+) ions appear necessary for the formation of these crystals.

The selectivity of the silver/copper reagent was tested against a number of controlled substances, including some with biological actions similar to GHB, i.e., tranquilization, amnesia induction, etc. Also tested were two compounds similar in structure to GHB, i.e., sodium salts of alpha-hydroxybutyrate and beta-hydroxybutyrate. As GHB encountered in casework is apt to be illicitly synthesized, the effectiveness of the reagent on mixtures of unreacted gamma-butyrolactone (GBL) precursor and GHB product was tested both before and after washing of the aqueous mixture with toluene. All tests were performed at an approximately neutral pH (6–8); strongly acidic solutions will degrade any GHB present and a strongly basic solution will form GHB from any GBL present. "Reagent crystals" (Fig. 2) were formed by the reagent alone on standing.

Sensitivity of the silver/copper reagent was tested against an approximately 125 mg/mL (1M) aqueous stock solution of GHB and serial dilutions.

A trial was performed using ten samples prepared by the author (Andera) and tested blind (Wojcik) using the described silver/copper microcrystal reagent. Solid samples were tested by dissolving them in water before combining them with the reagent. Included were commercially obtained GHB, GHB synthesized in the laboratory, and GHB submitted for forensic analysis by a client agency.

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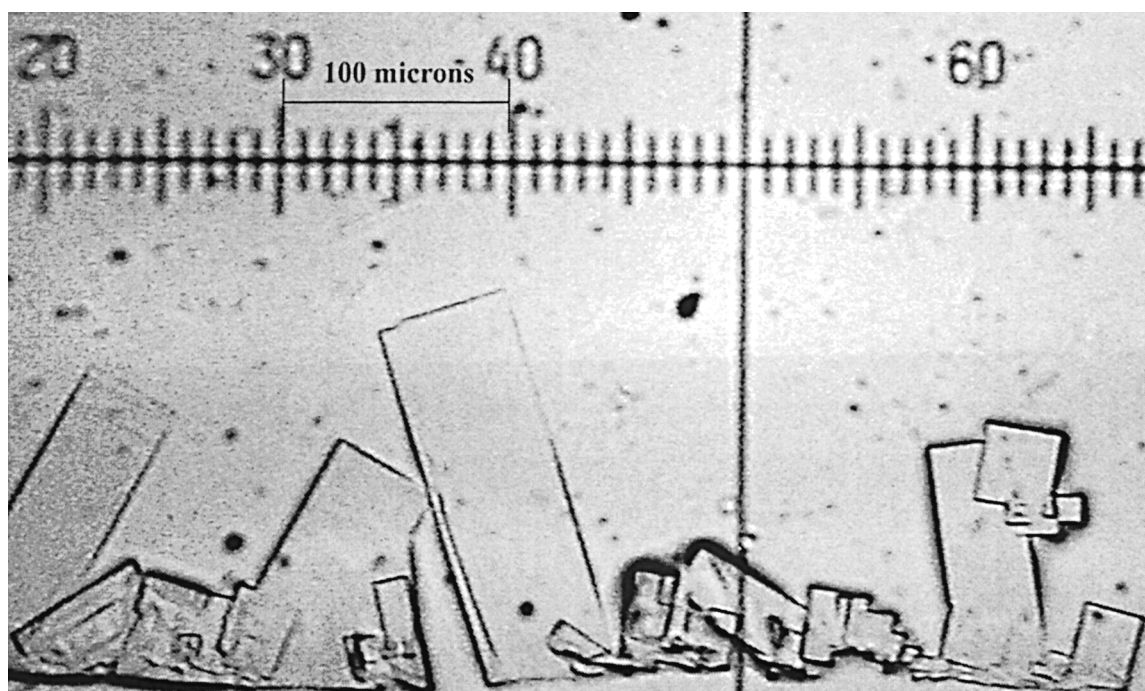


FIG. 1—Photomicrograph of crystal with GHB and silver/copper reagent.

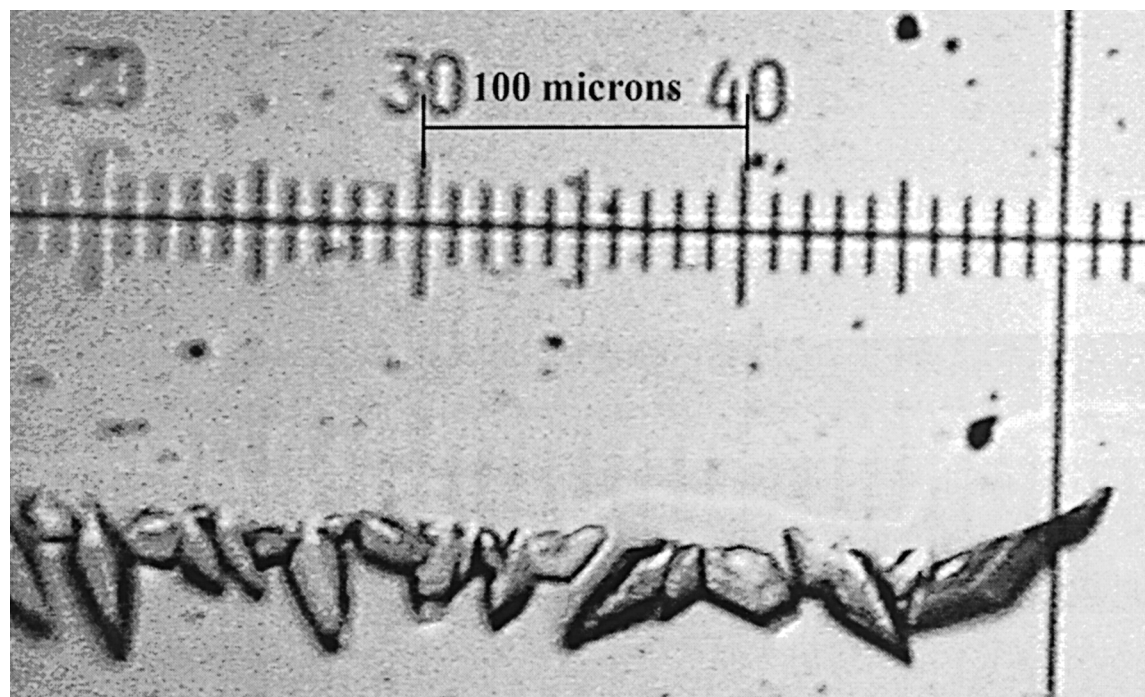


FIG. 2—Photomicrograph of silver/copper reagent crystal.

Reagents

All reagents were prepared using deionized water. Silver nitrate (Mallinckrodt Chemical Works; St. Louis, MO) and cupric nitrate (Aldrich Chemical; Milwaukee, WI) solutions were each prepared at a concentration of 1% w:vol. Cupric chloride/silver nitrate reagent was prepared from copper (II) chloride hydrate (Aldrich Chemical; Milwaukee, WI) and silver nitrate

to yield a 1% w:vol solution of cupric nitrate. Cupric nitrate/silver nitrate reagent was prepared from cupric nitrate and silver nitrate at a concentration of 1% each w:vol.

Drug Samples

Alpha-hydroxybutyrate, beta-hydroxybutyrate, GHB, and GBL were commercially obtained (Sigma Chemical; St. Louis, MO). An

additional GHB sample was synthesized (15–19) from sodium hydroxide (VWR Scientific; West Chester, PA) and GBL obtained from the San Bernardino County Sheriff's Department Narcotics Division with its identity confirmed by Fourier-Transform infrared spectroscopy (FT-IR) (10). A third GHB sample was obtained from evidence submitted by a client agency for forensic analysis. Its identity also was confirmed by FT-IR. Additional drug and controlled substance samples were obtained from SID's verified standards collection.

Apparatus

All samples were examined on glass microscope slides (Gold Seal Products; Portsmouth, NH) without a cover glass using an Olympus BH-2 polarizing microscope (Olympus America, Inc.; Melville, NY). Photomicrographs were taken using a Kodak DC-120 Zoom Digital Camera (Eastman Kodak; Rochester, NY) and images processed using Image Pro-Plus software (Media Cybernetics; Silver Spring, MD).

Results

The silver/copper nitrate reagent formed elongated crystals which grow from the edge of the drop, but are easily distinguished from the rectangular crystals resulting from GHB based upon their relief, birefringence colors when observed with polarized light, and retardation colors when examined with a first-order red plate.

Crystals developed in approximately 5 min with dilutions to approximately 4 mg/mL. No crystals developed within 10 min with an approximately 2 mg/mL dilution.

A mixture of 2.0 mL of precursor GBL was added to 125 mg/mL aqueous stock solution and tested with the reagent; no crystals formed within 10 min. One milliliter of the GBL/GHB mixture was extracted with an equal volume of toluene and the washed

TABLE 1—Silver/copper reagent specificity.

Drug	Result
Amytal	Reagent crystals
Aprobital	Clumps of amorphous crystals
Barbital	Reagent crystals
Butabarbital	No crystals
Chloral hydrate	Reagent crystals
Cocaine base	Dancing, amorphous crystals
5,5-Diallyl barbituric acid	Thin, triangular crystals
Ephedrine hydrochloride	No crystals, white precipitate
Flunitrazepam	Reagent crystals
Gamma butyrolactone (GHB precursor)	Needles
Heroin hydrochloride	No crystals, white precipitate
Hexobarbital	No crystals
Hydroxybutyric acid, Alpha-	Reagent crystals
Hydroxybutyric acid, Beta-	Clear, pointed spikes from edge and reagent crystals
Mephobarbital	Reagent crystals
Methamphetamine	Reagent crystals, whitish film
Methobarbital	No crystals
PCP hydrochloride	Reagent crystals, white precipitate
Pentobarbital	Hexagons, 3 dimensional
Phenobarbital	Reagent crystals
Probarbital	Reagent crystals, white precipitate
Secobarbital	Reagent crystals
Reagent control (blank)	Reagent crystals (see Fig. 2)

TABLE 2—Blind unknowns tested with silver/copper reagent.

Sample Number	Drug	Result
1	Cocaine base	No rectangular crystals Reagent crystals
2	GHB sodium salt, standard	Rectangular crystals Reagent crystals
3	Phenobarbital	No rectangular crystals Reagent crystals Rods and bushes
4	Flunitrazepam	No rectangular crystals Reagent crystals
5	5,5-Diallyl barbituric acid	No rectangular crystals Reagent crystals Clusters of rods
6	Beta-hydroxybutyric acid	No rectangular crystals Reagent crystals
7	Gamma-butyrolactone	No reagent crystals Diamond shaped crystals throughout
8	Water, de-ionized	No rectangular crystals Reagent crystals
9	GHB-solid	No rectangular crystals Reagent crystals Bushes growing from drop edge
10	GHB liquid	Rectangular crystals

aqueous layer was re-tested. The rectangular crystals typical of those for GHB developed in 3 min.

Table 1 shows the drugs and controlled substances tested and resulting crystals observed with the silver/copper reagent. Table 2 shows the unknowns and the results obtained by the analyst using the crystal reagent in the blind trial.

Discussion

An aqueous solution of 0.1 g cupric nitrate + 0.1 g of silver nitrate in 10.0 mL water can be used as a microcrystal test for GHB. The silver/copper reagent forms rectangular crystals at the drop periphery with GHB. It does not form these crystals with various other drugs, including some that have similar biological activity to GHB. Formation of the characteristic crystals with GHB occurs in approximately 5 min at concentrations down to approximately 2.0 mg/mL. Furthermore, silver ions in the reagent react with most negative ions to form an insoluble salt which may interfere with the formation or detection of GHB crystals. While no false positives were encountered in the testing, there was one false negative. Further experiments on verification by FT-IR of GHB in the rectangular crystals is planned.

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