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TECHNICAL NOTE

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Enhancement of Microcrystalline Identification of γ-Hydroxybutyrate*

ABSTRACT: An enhancement of the microcrystalline test for the detection of γ-hydroxybutyrate (GHB) is described. The original test used a silver/copper reagent which consisted of 0.1 g of silver nitrate and 0.1 g of copper nitrate in 10 mL water. The enhanced test utilizes lanthanum nitrate in place of copper nitrate. A detection limit of 0.5 mg/mL was achieved and the visual discrimination was improved because of larger sized crystals. Transient crystals were observed between 0.1 and 0.4 mg/mL. Silver nitrate alone appeared to be suitable for GHB detection but was not specific as other hydroxyl acids, such as glycolic acid, produced a similar crystal pattern. Tests conducted on chemical precursors of GHB and substances with similar biological activity highlight the specificity of the enhanced test. The reagent is therefore selective and sensitive for GHB in aqueous solutions. However, in beverage testing, crystal formation appeared to be inhibited for some drinks. Citric acid was identified as a possible interference depending on its concentration relative to GHB.

KEYWORDS: forensic science, γ-hydroxybutyrate, microcrystalline test, date rape drug, silver nitrate, lanthanum nitrate

γ-Hydroxybutyrate (GHB) is a small endogenous polar compound (Fig. 1). It has been a class C drug since 1 July 2003 in the United Kingdom and federally controlled (schedule I) since March 2000 in the United States of America. The protonated form of GHB is colorless and odorless and is a solid when converted into a metal salt. It has two chemical precursors: γ-butyrolactone (GBL) and 1,4-butanediol. *In vivo*, both undertake a rapid conversion into GHB via enzymatic processes (1). *In vitro*, GHB is converted into GBL in alkaline condition, and reversely in acidic conditions (2).

Because of its central nervous system depressant properties, GHB induces a range of effects from relaxation and euphoria, confusion, dizziness, nausea, short-term amnesia to uncontrollable shaking and seizures, respiratory depression, and unarousable unconsciousness (3). GHB has become one of the most frequently encountered drugs for abuse and malevolent purpose in the club drug scene and in date-rape cases (4).

Numerous analytical methods have been developed to identify it including: GC-FID (5), GC-MS (6), HPLC-UV and -MS (7), NMR (8), chemical/colorimetric spot tests (9), and LC-MS (10).

A microchemical identification of GHB was done using silver/copper nitrate reagent (11) showing distinctive crystal form when GHB is present. It was concluded that the reagent couple was selective, but not extremely sensitive. The detection limit was around 2 mg/mL. Investigating on ways to improve this test, several metal nitrates were tested on GHB and analogues. They were tested on their own and coupled with silver nitrate.

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Materials and Methods

Reagents and Dry Samples

All reagents were prepared in deionized water (18 MΩ).

Silver nitrate (99.9999%) was purchased from Sigma (Gillingham, Dorset, UK). Copper nitrate, sodium nitrate, mercuric nitrate, and cadmium nitrate were purchased from Sigma; zinc nitrate, ferric nitrate, potassium nitrate, calcium nitrate, and chromic nitrate from Fisons (Loughborough, Leicestershire, UK); ruthenium nitrate from ESPI (Ashland, Orlando, USA); and nickel nitrate, lithium nitrate, cobalt nitrate, and lanthanum nitrate from Merck (Poole, Dorset, UK). All reagents were prepared at 10 mg/mL concentration.

GHB sodium salt was purchased from Sigma and solutions ranging from 1000 mg/mL to 0.1 mg/mL were prepared. α-Hydroxybutyrate (AHB), β-hydroxybutyrate (BHB), and γ-butyrolactone (GBL) were obtained from Sigma and aqueous solutions containing 10 mg/mL were prepared.

All other drugs were purchased from Sigma and 10 mg/mL aqueous solutions were prepared.

Citric acid solutions were prepared with concentration ranging from 1 mg/mL to 40 mg/mL.

Method

All experiments were carried out with standard microscope glass slides (Menzel-glaser, Fisher [Loughborough, Leicestershire, UK]), and they were used as obtained. Microscopy was carried out using a Nikon Eclipse E800 microscope equipped with a Nikon Digital Net Camera DN100 (Kingston upon Thames, Surrey, UK).

Solutions of GHB were prepared across a range of concentrations and 10 μL was applied on a microscope glass slide (GHB mass range: 0.1–10 mg). Ten-microliter reagent solution was applied directly on top of the GHB drop. Separate 10 μL drops of reagent and GHB were also applied to the same slide as controls.

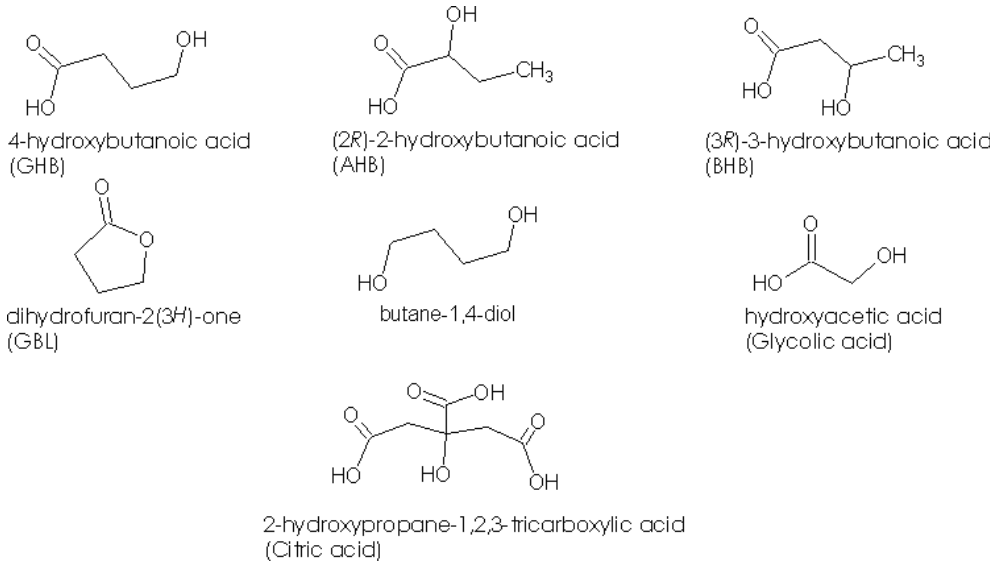

FIG. 1—*Chemical structures.*

After a few minutes (depending on the reagents and the GHB concentration) the slides were observed under a microscope with normal and polarized light at ×100 magnification. Crystal growth occurred initially at the periphery of the test area (within 5 min), and all across the area after complete drying (20 min maximum).

The drying was left to occur naturally at ambient temperature. The rate of crystal formation can be increased by heating the slides; however, this produces deformed crystals.

All precursors and control substances were tested using the above procedure.

Results and Discussion

It was found that $AgNO_3$ alone produces "*GHB right angle* crystals" when testing GHB (Fig. 2). Glycolic acid (Fig. 1) also produced similar shaped crystals. However, discrimination between GHB and glycolic acid was achieved when $AgNO_3$ was combined with 15 metal nitrates and tested on glycolic acid (no right angle crystals were observed). The ($AgNO_3$ + *metal nitrate*) combinations all produced "*GHB right angle crystals*" with GHB.

When utilized on GHB, $Cu(NO_3)_2$:$AgNO_3$(1:1) induced elongated crystals, which exhibit characteristic right angles and will be referred to as "*GHB right angle crystals*." This is in agreement with previous work (11).

From the 15 nitrates (Ca, Cd, Co, Cr, Cu, Fe, Hg, K, La, Li, Mg, Na, Ni, Ru, Zn) tested in combination with $AgNO_3$, lanthanum ($La(NO_3)_3$:$AgNO_3$[1:1]) induces the largest crystals and the best visual discrimination for a given concentration of GHB (Fig. 3). We have previously reported the successful use of this enhanced reagent (12,13) and it has been used and reported by other workers also (14).

$La(NO_3)_3$:$AgNO_3$(1:1) gives stable "*GHB right angle crystals*" when tested on GHB solutions with a range of concentrations down to 0.5 mg/mL. These crystals were observed after more than 5 weeks left at room temperature and exposed to air. Transient "*GHB right angle crystals*" (lifetime < 2 min) occurred between 0.1 and 0.4 mg/mL. No crystal formation was observed below 0.1 mg/mL. This is an enhancement of the previous reported detection limit for GHB of approximately 2 mg/mL with $Cu(NO_3)_2$:$AgNO_3$(1:1) (11).

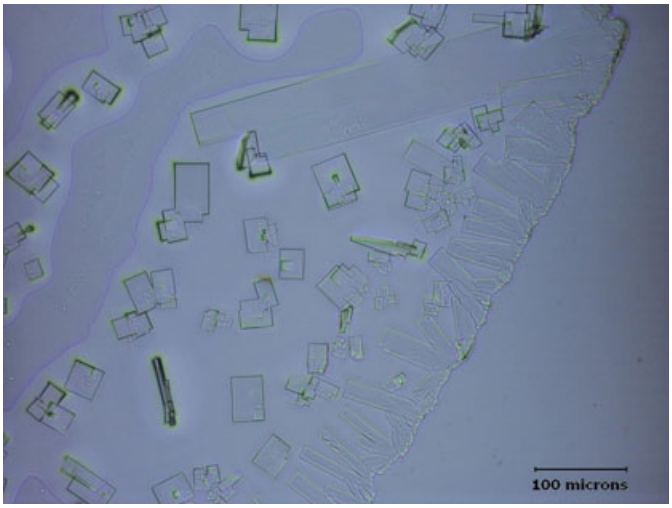

FIG. 2—*GHB + ($AgNO^3$).*

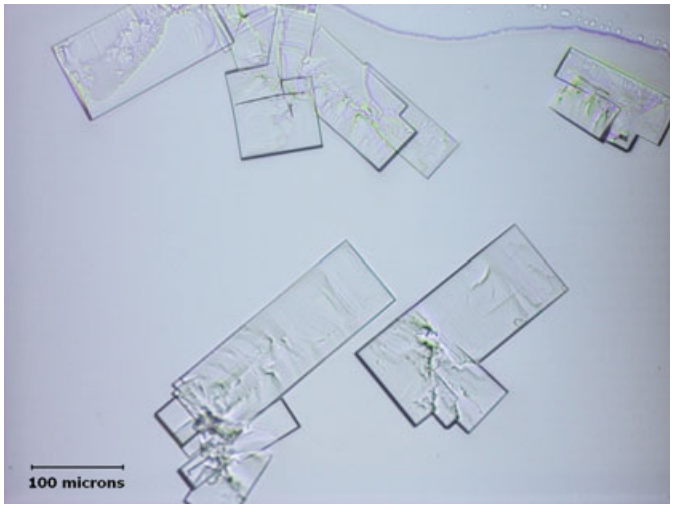

FIG. 3—*GHB + ($AgNO^3$: $La(NO^3)^3$ [1:1]).*

TABLE 1—*Reagent ($AgNO_3$:$La(NO_3)_3$ [1:1]) specificity.*

Substances	Observations	Presence of "right-angle" crystals
GHB	Right-angle crystals, reagent crystals	**Yes**
GBL	No crystal formation (very difficult to dry)	No
Chloral hydrate	Small reagent crystals	No
Methamphetamine	Brown color, colorless film	No
Pentobarbital	White halo precipitate, whitish film	No
D-amphetamine sulfate	Large crystals of random shape	No
Morphine	Four branch star shaped crystals, small triangular crystals, reagent crystals	No
Phenobarbital	White halo precipitate, iridescent halo background	No
Secobarbital	White precipitate, reagent crystals	No
Barbital	Very long blade-shaped crystals, right-angle crystals, reagent crystals	**Yes**
Diazepam	Small round crystals (droplets), reagent crystals, pale pink background	No
Flunitrazepam	Small pale green crystals with various shapes, reagent crystals	No
Oxazepam	Thin blade shape crystals with orange halo background, reagent crystals	No
Hexobarbital	Small pale green crystals with various shapes, reagent crystals	No
AHB	Yellow vast crystals with parallelepipedic edges	No
BHB	Needle shape crystals, transparent film on the edges	No
Reagents	Reagent crystals	No

GHB, γ-hydroxybutyrate; GBL, γ-butyrolactone; AHB, α-hydroxybutyrate; BHB, β-hydroxybutyrate.

GBL (Fig. 1) was tested but did not produce any crystals. $La(NO_3)_3$:$AgNO_3$(1:1) was tested on GHB isomers (AHB, BHB [Fig. 1]) and several other controlled substances (Table 1). No "*GHB right angle crystals*" were found for AHB and BHB. From the other substances, only barbital showed right angle crystals. When tested with $Cu(NO_3)_2$:$AgNO_3$(1:1), barbital also produced right angle crystal. However, barbital can be differentiated from GHB as the barbital control gave right angle crystals, whereas these were not observed with the GHB control.

$La(NO_3)_3$:$AgNO_3$(1:1) was tested on several drinks presenting a range of different challenging properties for the test. 10 mg/mL GHB was detected in Coke® and Sprite®, gin and vodka. However, the same concentration of GHB in beer, white wine, and various fruit juices did not yield any "*GHB right angle crystals*." As suggested previously (14), the thick viscosity of some drinks (mostly from the sugars present) could prevent the GHB crystal formation. Investigation on specific interferences has not previously been reported.

Competition between citric acid (Fig. 1) and GHB for the Ag^+ ions was investigated (Table 2). The citric acid concentration range covered concentrations expected to be found in most beverages (15). It was found that citric acid does inhibit "*GHB right angle crystal*" formation. For all concentration combinations tested, GHB concentration has to be equal or in excess of citric acid concentration to ensure "*GHB right angle crystals*" formation. As it is known that La(III) binds with organic acids (16), an increasing amount of $La(NO_3)_3$ (10 mg/mL up to 100 mg/mL) was added to $Cu(NO_3)_2$ in an attempt to complex the citric acid with lanthanum ions. The crystal formation pattern observed in Table 2 was unaffected. Henceforth, it is possible that a GHB dose, sufficient to cause impairment, would be masked by a high citric acid content and would go undetected using this enhanced reagent.

TABLE 2—*Citric acid interference on γ-hydroxybutyrate (GHB) crystal formation across concentration range (tested with $AgNO_3$/$La(NO_3)_3$).*

GHB (mg/mL)	Citric acid (mg/mL)							
	40	20	10	8	6	4	2	1
40	✔	✔	✔	✔	✔	✔	✔	✔
20	×	✔	✔	✔	✔	✔	✔	✔
10	×	×	✔	✔	✔	✔	✔	✔
8	×	×	✔	✔	✔	✔	✔	✔
6	×	×	×	✔	✔	✔	✔	✔
4	×	×	×	×	×	✔	✔	✔
2	×	×	×	×	×	×	×	✔
1	×	×	×	×	×	×	×	✔

✔ means presence of right angle crystals; × means absence of right angle crystals.

The results of this study demonstrate the limitation of the test in the detection of GHB in beverages. Further interference sources are under inspection.

Conclusion

A $AgNO_3$/$La(NO_3)_3$ reagent gives an enhanced detection of GHB by improving the visualization of GHB characteristic crystals and a lower detection limit in aqueous solution of 0.5 mg/mL.

GHB was positively discriminated among a range of GHB precursors, structure related compounds, and other control substances using the enhanced test.

The enhanced reagent does allow GHB detection in only a limited range of beverages. Citric acid was identified as an interferent that will produce false negative results when its concentration is greater than GHB.

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