#12
GHB, GBL, and 1,4 BD
Analytical Methods

1.0.0 Background

GHB (gamma-hydroxybutyrate) is a controlled substance in Idaho while its precursors GBL (gamma- butyrolactone) and 1,4 Butandiol (1,4 BD) are not. This is problematic in that the interconversion of GBL to GHB and 1,4 BD to GHB is simply pH dependant. In aqueous solutions GHB and GBL will exist in equilibrium, the relative concentrations of each are also pH dependent.

The following analytical scheme was developed to separate and identify GHB, GBL, and 1,4 BD while ensuring that GHB is not produced during the process.

2.0.0 Scope

The following analytical procedures are used to confirm the presence of GHB and its related analogs in samples. This method was based on a procedure found in "*Microgram, Vol XXXV, No.1 January 2002*".

3.0.0 Equipment and Reagents

The following pieces of equipment can be used to identify the analytes of interest.

3.1.0 A GC/MS and appropriate analytical software. Reference the GC/MS AM.
3.2.0 FTIR and appropriate analytical software. Reference the FTIR AM.
3.3.0 PH paper.
3.4.0 ACS grade chloroform, ethyl acetate, methanol, ethanol.
3.5.0 H_2SO_4, BSTFA (with 1% TMCS) a TMS derivatizing reagent. *NOTE the BSTFA is available premixed from Cerilliant Corporation.
3.6.0 Distilled or deionized water.
3.7.0 Bromocresol green, methyl orange, dextrose, aniline hydrochloride, sodium hydroxide. **NOTE, aniline is acutely toxic handle with care.**

4.0.0 Screening Tests

4.1.0 Color Spot Test

A mixture of Bromocresol green, Methyl orange, and Schweppes reagents are tested with samples. A positive reaction for the presence of GHB is one that turns green.

4.1.1 Bromocresol green
Mix 0.03g bromocresol green in 100 mL of 4:1 methanol: water. Adjust to pH 7 with NaOH.

4.1.2 Methyl Orange
Mix 0.01g of methyl orange in 100 mL of methanol. Adjust pH to 7.

4.1.3 Modified Schweppes
Solution A: mix 2.0g dextrose in 20 mL of water.

Solution B: mix 2.4g aniline hydrochloride in 20 mL of ethanol.
Mix solution A & B and dilute to 80 mL with methanol.

4.1.4 Mix Bromocresol green solution with the Methyl Orange solution in a 1:1 ratio. Add 3 parts of this combined solution to one part of the Schweppes reagent.

4.2.0 Physical tests.

4.2.1 Pure GBL and 1,4 BD are viscous liquids at room temperature. 1,4-BD will solidify when placed in a refrigerator (4^{o}C) while GBL will not.

4.2.2 GBL is soluble in chloroform and 1,4 BD is not.

4.3.0 GC/MS

Add concentrated Sulfuric acid to aqueous sample, extract with chloroform and analyze. If GBL is detected then proceed with confirmational GC/MS.

5.0.0 GC/MS Sample Preparation and Analysis

GHB cannot be analyzed directly on a GC/MS as it will convert to GBL in the heated injector port. GHB must be derivatized with BSTFA before injection.

5.1.0 1,4-Butandiol

5.1.1 If pure 1,4 BD is suspected then dilute with methanol and inject into GC/MS.

5.1.2 In aqueous samples, if the concentrations of 1,4 BD are high enough, then the 1,4 BD may be observed in a chloroform extract.

5.1.3 Dry down sample, add methanol, and analyze.

5.1.4 1,4 BD will derivatize with BSTFA as per 5.3.3.

5.2.0 GBL

5.2.1 Extract , or dilute if pure, with chloroform and analyze.

5.3.0 GHB

5.3.1 Extract aqueous samples with chloroform, discard chloroform.

5.3.2 Dry down aqueous layer with nitrogen or dry air. Sample can be warmed to expedite drying as long as the temperature remains below 60 C.

5.3.3 Once sample is <u>completely</u> dry then add 100-200 ul of BSTFA. Cap sample and heat at 60-70C for 15-20 minutes.

5.3.4 Add ethyl acetate and analyze on GC/MS.

6.0.0 FTIR

Aqueous samples are defined as clear, colorless liquids that appear to be water. This doesn't include sodas, sport drinks, etc.

6.1.0 1,4-BD.

If suspected to be pure, run as a liquid sample, i.e. liquid cell, salt windows

Gemini, ATR etc.

6.2.0 GBL
- 6.2.1 If pure, then analyze as a liquid.
- 6.2.2 If aqueous, extract with chloroform. Discard aqueous layer. Evaporate off chloroform and run as a liquid.

6.3.0 GHB
- 6.3.1 If solid, analyze as a KBr pellet.
- 6.3.2 If aqueous, extract with chloroform. Discard chloroform. Evaporate to dryness and run as a KBr pellet.

7.0.0 Scheme

7.1.0 Solids.
- 7.1.1 Run color test
- 7.1.2.1 If color test is negative, dissolve in Methanol and analyze on GC/MS.
- 7.1.2.2 If color test is positive, skip to 7.1.4.
- 7.1.3 If GC/MS is negative then analysis is complete.
- 7.1.4 If GC/MS has GBL then derivatize original sample with BSTFA and analyze on GC/MS as per sections 5.3.3 and 5.3.4. Or run sample on FTIR.

7.2.0 Clear, thick liquids.
- 7.2.1 Place 1-5 mls of the sample in the freezer for fifteen minutes. If it solidifies, extract with methanol and analyze with GC/MS. If results indicate the presence of GBL proceed to section 7.3.4 and 7.3.5.
- 7.2.2 If sample remains a liquid go to section 7.3.0.

7.3.0 Aqueous samples.
- 7.3.1 Perform color test.
- 7.3.2 Acidify a portion of the sample with concentrated H_2SO_4 and extract with chloroform. Analyze the chloroform layer with GC/MS. If results are negative for GBL then proceed with section 7.3.3. If GBL is present then skip to section 7.3.4. If 1,4 BD is present then report.
- 7.3.3 If results from 7.3.2 indicate the presence of 1,4 BD then report. If results were negative then take a portion of original sample and dry down with nitrogen and heat (60C). Extract with methanol and analyze with GC/MS.
- 7.3.4 Take a portion of original sample extract with chloroform. Analyze chloroform layer with GC/MS. Report GBL if found.
- 7.3.5 Take aqueous layer from 7.3.4 and analyze using sections 5.3.2 through 5.3.4.

8.0.0 History

Revision #	Issue or review date	History	Author or Reviewer
0	9/25/03	Original Issue	D.C. Sincerbeaux
1	1/12/07	Added page #'s& history changed sec 3.	D.C. Sincerbeaux
2	7/3/2007	Changed 3.4,3.5,3.6,3.7 reagent grades and Scope	D.C. Sincerbeaux