K50 Validation of a Color Test for Gamma- Hydroxybutyrate and Gamma-Butyrolactone

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After attending this presentation, attendees will have a better understanding of the ferric hydroxamate color test for the detection of gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL).

This presentation will impact the forensic science community by serving to provide a validated effective screening/presumptive color test for the detection of GHB in evidential samples.

GHB is one of the most widely-used drugs for drink adulteration in "date-rape" cases. Several test kits have been introduced to detect GHB in drinks; however, presently there is no single effective color test available for use by forensic drug laboratories to screen evidential submissions for GHB and GBL. Previously, Michalik et al.[1] described the creation of a new colorimetric spot reagent for the detection of GHB and GBL. This colorimetric spot reagent was an adaptation of the ferric- hydroxamate test for lactones.[2] It is a simple test that requires little sample preparation, and takes just a few seconds to accomplish. The test is able to detect 1 mg/mL of GHB or GBL in solid-dosage submissions as well as various matrices such as soft drinks and alcoholic beverages. This presentation will highlight the data and tests conducted to validate the hydroxamate colorimetric spot test for the detection of GHB and GBL in different matrices.

This work was conducted in order to determine how well the ferric hydroxamate test would be able to detect GHB and GBL in a variety of matrices including beverages. The procedure is a four-step procedure: (1) addition of 1 drop of concentrated H_2SO_4, (2) 3 drops of 0.5M hydroxylamine HCl in 95% ethanol/6M NaOH, (3) 1 drop of concentrated HCl, (4) 1 drop of 5% $FeCl_3$. Treatment of solutions of GHB and/or GBL with the reagents in this successive procedure produces a deep magenta color immediately. The magenta color formed with a positive reaction was distinctly different from the color of a negative reaction, which produces a brown precipitate or a ferric chloride solution, which was light yellow in color. Using commercially purchased synthetic GHB in pure deionized water, the hyrdroxamate color test gave a positive magenta color response down to 1 mg/mL. No false negatives were observed. All tests were conducted on the matrix, a water blank (deionized H_2O, concentrated H_2SO_4, and reagents), reagent blank (reagents only), and a matrix blank (matrix, reagents, and concentrated H_2SO_4). Potential interferents and water gave a similar response. Experiments with beverages containing GHB focused on allowing spiked and unspiked beverages to be compared directly.

Matrices tested included water (tap and bottled), coffee (regular and decaf), cranberry juice, orange juice, sprite, coca-cola, lime juice, lemon juice, pineapple juice, an energy drink, mouthwash, and various alcoholic beverages including several different wines, beers, and liquors. Analytes tested were GHB, GBL, 1,2-butanediol, 1,3-butanediol, 1-4- butanediol, 2,3-butanediol, beta-butyrolactone, gamma-valerolactone, caprolactone, dihydrocoumarin, dextromethorphan hydrobromide, caffeine, ephedrine, papaverine, cocaine, diazepam, and methamphetamine. It should be noted that the only other compounds tested that gave a false positive were other lactones (gamma- valerolactone caprolactone, dihydrocoumarin, and betabutyrolactone). This is to be expected since the test is specific for lactones, but is not of concern since these compounds are normally not encountered in GHB submissions. Of particular note, the test is negative for alcoholic beverages tested except for the red wines which were difficult to interpret due to the color of the solution.

The color formation with the ferric-hydroxamate reagents constitutes a highly specific screening test for GHB and/or GBL in a variety of matrices normally encountered in a forensic environment. The low cost of the reagents and their apparent reliability suggest that the test would be a useful screening tool for forensic scientists in the crime laboratory as well as officials investigating rape cases.

References:

1. K Michalik and TA Brettell, Abstract #B71, 60th Annual Meeting of the AAFS, Washington, DC, February 22, 2008.
   WC Alston and K Ng Forensic Sci. Int. 2002; 126(2002):114-117.

Forensic Science, GHB, GBL

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