B172 Extractionless GC/MS Analysis of γ-Hydroxybutyrate and γ-Butyrolactone With Trifluoroacetic Anhydride and Heptafluoro-1-butanol From Aqueous Samples

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This presentation will describe the development of a technique designed for qualitative and quantitative determination of γ-Hydroxybutyrate (GHB) and indirectly γ-Butyrolactone (GBL) from aqueous matrices without the need for an organic extraction. GHB is derivatized in the presence of water without the need for an organic extraction. This technique offers several advantages such as rapid determination, minimum sample handling, low sample volume, high sensitivity, improved mass spectra for confirmation and indirect determination of GBL concentration.

This may become a general technique in both the toxicology arena and the drug testing community. The technique offers minimal sample handling and excellent detection of a commonly abused drug in our society.

γ-Hydroxybutyrate (GHB) is a DEA Scheduled I drug of abuse commonly spiked into beverages to incapacitate victims of sexual assault. GHB is a challenging drug for analysis by GC/MS because of its small size, charged nature, low volatility and intramolecular esterification leading to γ-butyrolactone (GBL). In this work an extractionless technique has been developed that allows for the use of an aqueous sample for direct derivatization. The technique uses a solution of trifluoroacetic anhydride (TFAA) and 2,2,3,3,4,4,4-heptafluoro-1-butanol (HFB) to derivatize the active hydrogens of GHB. The conversion of GBL to GHB can be forced under alkaline conditions by diluting the sample in 10 mM borate buffer, pH 12.0. Legally GBL found in beverages intended for human consumption is considered a scheduled substance under current analogue law. Spikes of the two compounds into several beverage matrices gave quantitative recovery of GHB by GC/MS. The derivatization produces higher molecular mass products whose fragmentation pattern provides multiple peaks for confirmation and quantitation. The concentration of GBL can also be indirectly determined by the method developed by analyzing an aliquot of the same sample under hydrolytic conditions and in water. Therefore, this extractionless technique is rapid, sensitive and selective for the confirmation of the presence GHB and GBL in commercial beverages.

The current study shows the utility of an extractionless technique for the determination of GHB. The technique can be as simple as taking an aqueous neat sample and performing the derivatization. The dilution with borate buffer is only necessary if the conversion of any GBL to GHB is desired by hydrolysis of the lactone. The derivatization was monitored by full-scan mode to choose the ions with best sensitivity and selectivity. This derivatization technique produces sufficient high abundance fragments to make the identification by selected ion monitoring mode (SIM) easier than derivatizations that produce few and low abundance ions such as BSTFA/1%TMCS derivatives of GHB. Both the esterification and trifluoroacetylation are products of this reaction.

The standard curve has a linear range of 0 to 100 ppm with a correlation coefficient, r = 0.999. A ratio of standard (GHB) to internal standard (GHB-d_6) response was used to correct for variations in the derivatization and chromatographic processes. A wide range 0 to 1000 ppm standard curve gave a coefficient of correlation of r = 0.996. This analysis has a limit of detection (S/N=3) of 50 ppb and a limit of quantitation (S/N=10) of 150 ppb. The precision of the assay is excellent with percent coefficient of variation (%CV) below 5%.

Spike and recovery studies were performed with some common neat beverage matrices with and without alcohol. The recoveries are within 11% of the target value. This is impressive since the sample matrix was not diluted. In addition, the neat rum spiked sample contains approximately 40% ethanol. The next set of studies involved spiking both GHB and GBL into the different matrices and comparing their recoveries against the GHB standard curve. In this set of experiments the 1:10 dilution in 10 mM borate buffer, pH 12 was used to force the hydrolysis of GBL to GHB. GHB recoveries were on average about 100% and the GBL+GHB spiked matrices were on average 106%. The use of selected ion monitoring (SIM) mode for the quantitative analysis increases sensitivity over full-scan mode. The high concentrations encountered in typical beverages can easily allow the analysis to be performed in full-scan mode. The estimation of GBL concentration present in an aqueous solution can also be indirectly determined by submitting an aliquot of the sample to the alkaline hydrolysis and another aliquot to a simple dilution in water. The difference between the two values is negligible if the substance present is only GHB. If GBL is present than the two values will differ accordingly. Several urine matrix spikes were performed and the recoveries were within 15% of the target spiked values. Therefore, this assay may also be used for the determination of GHB in biological matrices.

Analysis of compounds with similar chemical structures included: 4-aminobutyric acid (GABA), diethylene glycol, 1,4-butanediol and gamma-butyrolactone (GBL). These were derivatized and monitored to account for any contribution to the GHB response. GBL was tested without the hydrolysis conversion step of the method to check the percent that converts to GHB in the process. The materials were spiked to a maximum of

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ten times the upper GHB standard curve concentration. The spiked concentration of the test compound was read from the GHB standard curve and the percent response was calculated from the fitted regression slope of the compound across the range tested. The only compound that gave a significant GHB peak was GBL. The GBL response was approximately 10% under these conditions across a wide range.

Some of the benefits of this method are the lack of an extraction step, very small sample size, the rich mass spectra obtained upon derivatization providing many abundant ions available to monitor by SIM mode, increased sensitivity and ability to detect both GHB and GBL in a complex beverage matrix. The typical analysis involves a significant dilution to get the concentration in the range of the assay. This dilution can be performed with either deionized water or the hydrolyzing borate buffer. The detection of either GHB or GBL in beverages is important in the forensic community because of the legal ramifications and the intended illegal use of GBL as an analogue of GHB. In vivo GBL is converted to GHB thus making the presence in beverages an illegal controlled substance similar to GHB. The conversion step of GBL to GHB allows for chemist not to miss the current trend of spiking GBL in beverages intended for human consumption. The technique requires minimal sample handling and provides reliable quantitation of GHB and GBL.

GHB, GBL, Extractionless

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