Determination of Gamma Hydroxybutyrate (GHB) in Hair Samples Using Solid Phase Extraction and LC-MS/MS

UCT Part Numbers:

CUQAX156: CLEAN-UP® Quaternary Amine w/ Chloride Counter Ion 500mg/6mL

September 2013

Introduction

Gamma-Hydroxybutyrate (GHB) is a naturally occurring substance found in the human central nervous system. It is also easily synthesized. GHB has been used medically as a general anesthetic, as well as to treat conditions such as insomnia, clinical depression, narcolepsy, and alcoholism. Illegally, it has been used as an intoxicant or as an agent in Drug Facilitated Sexual Assaults (DFSA) or to improve athletic performance. GHB has a very short window of detection in urine and blood making it difficult to detect in support of date rape cases. Using hair samples has been shown to be a viable alternative for detecting the presence of GHB above normal levels. This application note describes the extraction and subsequent analysis of GHB from decontaminated hair samples. After incubation in methanol the extract is dried; then dissolved in deionized water prior to sample clean up using Anion Exchange SPE (**CUQAX156**) followed by LC-MS/MS analysis.

Procedure

1. SAMPLE PREPARATION

a) To a clean glass tube add 100 mg of decontaminated hair sample.

b) Add 1 mL of CH_3OH and internal standard*, vortex mix

c) Incubate at 40 °C for approx. 12 hours

d) Centrifuge sample at 3000 rpm for 10 minutes

e) Transfer organic phase to a clean glass tube

f) Evaporate to dryness < 40 °C

g) Dissolve residue in 3 mL of DI H_2O (pH 7)

h) Vortex Mix

2. CONDITION EXTRACTION COLUMN

a) 1 x 3 mL CH_3OH

b) 1 x 3 mL D.I. H_2O

NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying

3. APPLY SAMPLE

a) Load sample at 1 to 2 mL/minute

4. WASH COLUMN

a) 1 x 3 mL DI H_2O

b) 1 x 3 mL CH_3OH

c) Dry column (10 minutes at > 10 inches Hg)

5. ELUTE GHB

a) 2 x 3 mL CH_3OH containing 6% Acetic Acid

b) Collect eluate at 1 to 2 mL / minute

6. DRY ELUATES

a) Evaporate to dryness under nitrogen < 40°C

b) Reconstitute in 100 µL of mobile phase

Instrument Conditions

Column: Thermo Fisher Gold C18; 50mm x 2.0 mm (1.9 µm)

Column Temperature: 40º C

Mobile phase: Acetonitrile w/ 0.1% Formic acid: D.I. H_2O w/ 0.1% Formic acid; (50:50)

Injection volume: 10 µL

Flowrate: 0.2 mL / minute

Detector: API 4000 MS/MS

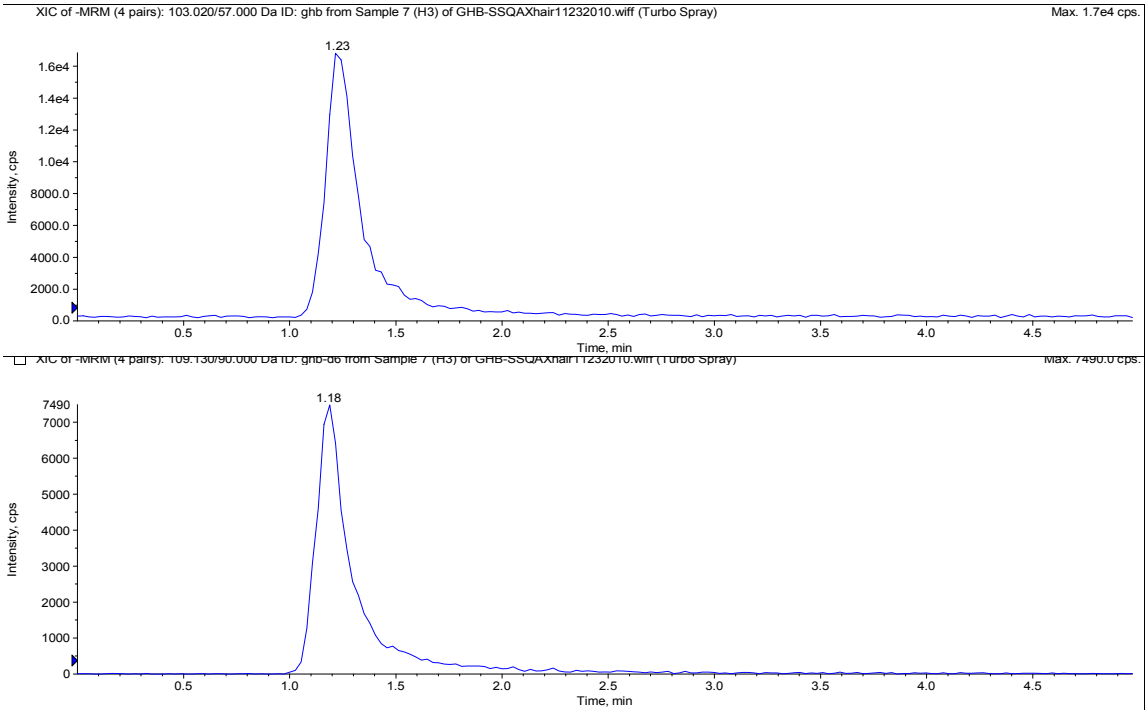
Ion Source	ESI
Ion Mode	Negative
Ion Spray Voltage	- 4500V
Curtain Gas	10
Gas 1	40
Gas 2	40
CAD Gas	Medium
Source Temp	650 °C
Mode	Positive

Mass Spec Table

Compound	RT (min)	Q1	Q3	DP	EP	CE	CXP
GHB (1)	1.23	103.0	57.0	-20	-8	-18	-8
GHB (2)	1.23	103.0	84.0	-20	-8	-14	-8
GHB-D6 (1)	1.18	109.1	90.0	-20	-8	-18	-4
GHB-D6 (2)	1.18	109.1	60.9	-20	-8	-18	-4

Note: Q1 = Precursor Ion; Q3= Product Ion; DP= Declustering Potential; EP= Entrance Potential; CEP= Collision Entrance Potential; CE= Collision Energy; CXP= Collision Exit Potential.

Chromatogram of GHB (upper trace) and GHB-D6



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