A Reduced Workflow Solution for the Analysis of *Gamma*-Hydroxybutyrate (GHB) in Human Hair Samples Via an Automated Bead Mill as a Precursor to GCxGC-TOFMS and GC-HRT

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Introduction

Background

Gamma-hydroxybutyrate (GHB) is an endogenous compound and is also a drug commonly used by bodybuilders, observed in the club scene, and used in drug-facilitated sexual assaults. GHB has been shown to have an elimination rate of 18 mg/L/h, so it is typically cleared from the blood within approximately 6 hours and from the urine within 12 hours after it is taken orally. Hair samples have proven valuable in detecting exposure to drugs of abuse over extended time periods.

Some of the analytical challenges associated with hair analysis include:

- Complexity of the Hair Matrix
- Chemical Diversity of Compounds in Hair
- Coelution of Analytes (e.g., **Urea and GHB**)
- Concentration Range of Sample Constituents
- Inappropriate Sample Preparation Methods
- Unsuitable Analysis Protocol

The Solution

Improved Sample Preparation (Bead® Ruptor 24)

GCxGC-TOFMS —Pegasus 4D (Enhanced Chromatographic Resolution)

- Increased Peak Capacity = Enhanced Chromatographic Resolution for Separation of Coeluting Analytes
- High Quality Spectral Data
- Comprehensive
- Search Against Well-Established Databases (e.g., NIST, Wiley)

Pegasus® 4D GCxGC-TOFMS

GC-HR TOFMS —Pegasus GC-HRT (Enhanced Mass Spectral Resolution)

- High Quality Spectral Data (Search Well-Established Databases such as NIST and Wiley)
- Excellent Mass Accuracy Values (<1 ppm) = Robust Formulas for Fragment, Molecular, and Adduct Ions
- High Resolution Deconvolution™ (HRD™)
- High Resolving Power (up to 50,000) = Increased Selectivity for:
  - Discovery
  - Confirmation
  - Comprehensive Profiling of Complex Samples

Pegasus GC-HRT

Experimental

Sample Preparation Methods

Extraction

- Head hair samples were obtained from volunteers (A and B) and washed:
  - 3x with DI-H_2O with sonication for 5 minutes per wash
  - 3x with CH_2Cl_2 with sonication for 5 minutes per wash
- Dried between pieces of filter paper overnight
- Split into two sets:
  - Set 1 was homogenized using a Biotage Bead Ruptor 24 (A1, A2; B1, B2)
  - Set 2 was cut into 1-2 mm segments using scissors (A3, A4; B3, B4)
- Two portions (20 mg each) were placed in microcentrifuge tubes
- A blank and 5 calibrators of concentrations 0.15-5.0 ng/mg were also placed into microcentrifuge tubes
- 1 mL of CH_3OH and 50 µL GHB-*d6* (1 µg/mL) were added to each tube
- Samples were incubated overnight at 40°C with agitation, and then dried with N_2(g)

Derivatization

100 µL of BSTFA was added to each sample and calibration standard. The reaction mixtures were heated at 60°C for 1 hour. The resulting products were transferred to 2 mL GC vials for analysis.

Instrument Parameters (Modulation applies only to GCxGC)

Gas Chromatograph	Agilent 7890, Dual Stage Quad Jet Modulator and MPS2 Autosampler
Injection	1 mL, Pulsed Splitless @ 250°C
Carrier Gas, Flow	He @ 1.5 mL/min, Corrected Constant Flow
Column One	Rxi-5MS, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Column Two	Rtx-200, 1.25 m x 0.20 mm x 0.25 µm coating (Restek)
Temp. Program	70°C (1 min) to 320°C @ 8°C/min (10 min); primary oven maintained +10°C relative to secondary oven
Modulation (Peg 4D only)	4s with temp. maintained +15°C relative to secondary oven
Transfer Line	300°C
Mass Spectrometers	**LECO Pegasus HT (unit mass resolution) and GC-HRT (R = 25,000)**
Ion Source Temp.	250°C
Mass Range (m/z)	45–510
Acquisition Rates	200 spectra/s for GCxGC; 10 spectra/s for GC-HRT

GCxGC-TOFMS Results (Enhanced Chromatographic Resolution)

Derivatized GHB and urea were easily separated using GCxGC-TOFMS technology as illustrated for sample B1 in the surface plot expansion below (Figure 1). This resulted in high quality spectra for GHB and facilitated quantitation (Figure 2, Table 1).

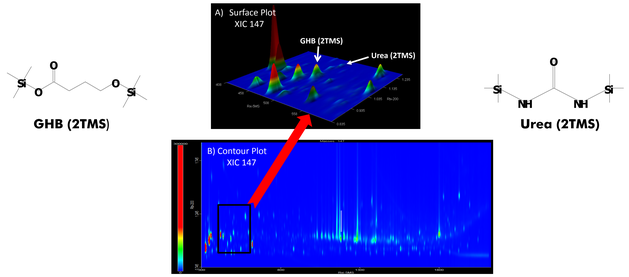
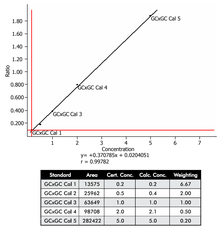


Figure 1: GCxGC-TOFMS (XIC, m/z 147) Surface Plot Expansion of the Contour Plot (B) Showing Separation of GHB and Urea



Standard	Area	Cert. Conc.	Calc. Conc.	Weighting
GCxGC Cal 1	13575	0.2	0.2	6.67
GCxGC Cal 2	25962	0.5	0.4	2.00
GCxGC Cal 3	63649	1.0	1.0	1.00
GCxGC Cal 4	98708	2.0	2.1	0.50
GCxGC Cal 5	282422	5.0	5.0	0.20

Figure 2: GCxGC-TOFMS Calibration Curve for GHB

Table 1. GHB in Samples A1-A4

Sample	Conc. (ng/mg)
A1 (Bead)	0.30
A2 (Bead)	0.33
A3 (Cut)	0.22
A4 (Cut)	0.24

Sample	Conc. (ng/mg)
B1 (Bead)	1.08
B2 (Bead)	0.98
B3 (Cut)	0.74
B4 (Cut)	0.80

Hair extracts consisted of a wide variety of compounds. A small representative set of compounds in sample B1 are listed in Table 2 (Ave. spectral similarity = 879/1000). Included in this list are flavor/scent (soleron, versalide), insect repellent (Deet), and sun screen (octocrylene, homosalate) components.

Table 2. Representative Compounds in Sample B1

Name	Formula	R.T. (s)	Area	Similarity
Soleron	$C_7H_8O_2$	400 , 2.660	72868	893
2-Furoic Acid (TMS)	$C_8H_{12}O_3Si$	408 , 1.230	1186413	908
Levulinic Acid (TMS)	$C_8H_{14}O_3Si$	408 , 1.445	264896	948
Tarragon	$C_{11}H_{14}O$	468 , 1.070	39438	913
Malonic Acid (2TMS)	$C_9H_{20}O_4Si_2$	476 , 1.265	62241	871
3-Hydroxycapricic Acid (2TMS)	$C_{19}H_{40}O_3Si_2$	576 , 1.030	44804	804
Phenoxyethanol (TMS)	$C_{11}H_{18}O_2Si$	624 , 1.065	192968	925
Thymine (2TMS)	$C_{11}H_{20}N_2O_2Si_2$	664 , 1.035	17474	805
Parabanic Acid (2TMS)	$C_9H_{16}N_2O_3Si_2$	732 , 1.925	90088	842
Adipic Acid (2TMS)	$C_{12}H_{24}O_4Si_2$	756 , 1.230	412706	900
5-Oxoproline (2TMS)	$C_{11}H_{23}NO_3Si_2$	772 , 1.570	255386	901
Cinnamic Acid (TMS)	$C_{12}H_{16}O_2Si$	788 , 1.215	9561	813
Diethyltoluamide	$C_{12}H_{17}NO$	816 , 1.725	351502	928

Name	Formula	R.T. (s)	Area	Similarity
Pimelic Acid (2TMS)	$C_{13}H_{28}O_4Si_2$	840 , 1.220	110224	899
3-Hydroxycapric Acid (2TMS)	$C_{19}H_{42}O_3Si_2$	884 , 1.010	8466	811
Propylparaben (TMS)	$C_{13}H_{20}O_3Si$	888 , 1.250	34179	878
Butylparaben (TMS)	$C_{13}H_{20}O_3Si$	968 , 1.235	207132	876
Azelaic Acid (2TMS)	$C_{15}H_{32}O_4Si_2$	992 , 1.200	89210	901
Versalide	$C_{15}H_{22}O$	1040 , 1.105	37772	822
Homosalate (TMS)	$C_{16}H_{25}O_3Si$	1164 , 1.165	2292399	922
Oleic Acid (TMS)	$C_{21}H_{42}O_2Si$	1260 , 1.020	3282718	880
9Z-Octadecen-1-ol (TMS)	$C_{21}H_{44}OSi$	1352 , 0.925	1012588	879
Monopalmitin (2TMS)	$C_{25}H_{52}O_4Si_2$	1508 , 1.025	178351	840
Batyl Alcohol (2TMS)	$C_{27}H_{58}O_3Si_2$	1556 , 0.920	742264	901
Octocrylene	$C_{24}H_{27}NO_2$	1556 , 1.410	1174756	922
Cholesterol (TMS)	$C_{30}H_{54}OSi$	1800 , 1.055	15472352	882

GC-HRT Results (Enhanced Mass Spectral Resolution)

GC-HRT analysis produced high-resolution, deconvoluted spectra that were searched against large databases (NIST, Wiley) and accurate mass ions that were leveraged in molecular and fragment ion formula determinations. An Analytical Ion Chromatogram (AIC) for sample A1 is displayed in Figure 3. Names, formulas, retention times, similarities, and mass accuracy values for a representative set of compounds in A1 are listed in Table 3.

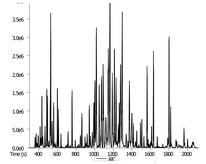


Figure 3: GC-HRT AIC for A1

Table 3. Representative Compounds in Sample A1

Name	Formula	R.T. (s)	Area	Similarity	Ion	MA (ppm)
UREA (2TMS)	$C_7H_{20}N_2OSi_2$	486	12127269	928	$M^{+\cdot}$	-0.24
Benzoic Acid (TMS)	$C_{10}H_{14}O_2Si$	492	5448582	912	$M^{+\cdot}$	-0.32
Methylmalonic Acid (2TMS)	$C_{10}H_{24}O_4Si_2$	559	10518372	950	$M^{+\cdot}$	0.67
Serine (3TMS)	$C_{12}H_{31}NO_3Si_3$	611	4989159	938	$[M-CH_3OSi]^+$	-0.40
4-Hydroxybenzaldehyde (TMS)	$C_{10}H_{14}O_2Si$	614	125596	846	$M^{+\cdot}$	0.96
5-oxo-Proline (2TMS)	$C_{11}H_{23}NO_3Si_2$	757	20156041	936	$M^{+\cdot}$	0.32
Phenylalanine (2TMS)	$C_{15}H_{25}NO_2Si_2$	849	3862385	883	$[M-C7H7]^+$	-0.03
Myristic Acid (TMS)	$C_{17}H_{34}O_2Si$	976	3647162	841	$M^{+\cdot}$	-6.09
Uric Acid (4TMS)	$C_{17}H_{36}N_4O_3Si_4$	1222	277571	789	$M^{+\cdot}$	0.63
9Z,12Z-Octadecadienoic Acid (TMS)	$C_{21}H_{40}O_2Si$	1266	3539559	784	$M^{+\cdot}$	-0.66
Stearic Acid (TMS)	$C_{21}H_{44}O_2Si$	1298	32507255	904	$M^{+\cdot}$	-0.83
9-Octadecenamide	$C_{18}H_{35}NO$	1375	26423908	844	$M^{+\cdot}$	-0.65
1-Monomyristin (2TMS)	$C_{23}H_{50}O_4Si_2$	1398	2094427	828	$[M-CH_3OSi]^+$	-0.03

ChromaTOF-HRT® brand software was used to process data for qualitative and quantitative analysis. The calibration curve for GHB (internal standard = GHB-*d6*) was constructed using quantitation and reference ions m/z = 233.1024 and 239.1400 (Figures 4-6). Concentrations of GHB in A and B hair samples are listed in Table 4.

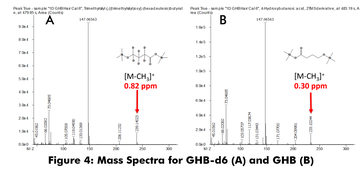


Figure 4: Mass Spectra for GHB-d6 (A) and GHB (B)

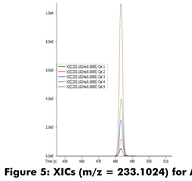
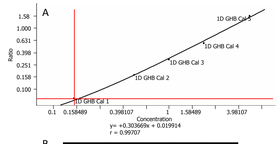


Figure 5: XICs (m/z = 233.1024) for A1-A4



Standard	Area	Cert. Conc.	Calc. Conc.	Weighting
1D GHB Cal 1	12048	0.2	0.2	6.67
1D GHB Cal 2	28695	0.5	0.5	2.00
1D GHB Cal 3	60094	1.0	0.9	1.00
1D GHB Cal 4	95763	2.0	1.8	0.50
1D GHB Cal 5	257491	5.0	5.2	0.20

Figure 6: GC-HRT Calibration Curve for GHB

Table 4. GHB in Samples A1-A4 and B1-B4

Sample	Conc. (ng/mg)	MA (ppm)
A1 (Bead)	0.41	-1.16
A2 (Bead)	0.61	-0.06
A3 (Cut)	0.28	-0.79
A4 (Cut)	0.31	0.81

Sample	Conc. (ng/mg)	MA (ppm)
B1 (Bead)	1.34	-0.18
B2 (Bead)	1.21	1.20
B3 (Cut)	1.08	0.29
B4 (Cut)	1.04	-0.62

Discussion

Hair extracts consisted of a complicated mixture of compounds transferred internally from blood or externally from sources such as conditioner, sun screen, or other commercial products. Greater quantities of GHB were extracted via the "Bead" rather than "Cut" sample preparation methods (Table 4). In addition, more GHB was found in hair from volunteer B.

Conclusion

- The Biotage Bead Ruptor 24 was effective in pre-treating hair samples prior to the extraction procedure.
- The enhanced chromatographic resolution of GCxGC-TOFMS and high resolving power of GC-HRT minimized interferences and facilitated accurate analysis of GHB in hair.