Quantitative Analysis of γ-Hydroxybutyrate in Hair Using Target Analyte Finding Processing of Comprehensive GC-HRT Data

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Key Words: Pegasus® GC-HRT, GHB, Hair, Target Analyte Finding, Quantitative Analysis

1. Introduction

Hair sample analysis has been proven to be very valuable in detecting exposure and providing an indication level of drug abuse over extended periods of time. In this study, two sample preparation methods were tested for their effectiveness in extracting gamma-hydroxybutyrate (GHB) from hair samples. GHB is an endogenous compound and is also a drug commonly used by bodybuilders. Additionally, it has been observed in the club scene, and used for drug-facilitated sexual assaults. GHB has been shown to have an elimination rate of 18 mg/L/h, so it is typically cleared from blood within 6 hours and from the urine within 12 hours after oral ingestion. Fortunately, it remains in hair over extended periods of time and can be easily detected using gas chromatography high resolution time-of-flight mass spectrometry (GC-HRT) as shown in Figure 1. This application note will demonstrate the use of the Target Analyte Finding feature of the ChromaTOF-HRT® brand software for fast quantitative determination of targeted analytes. In addition, non-targeted analytes will also be discussed since the comprehensive nature of TOFMS analyses paired with ChromaTOF-HRT's High Resolution Deconvolution® (HRD®) allows target and non-targeted compounds to be determined in a single acquisition.

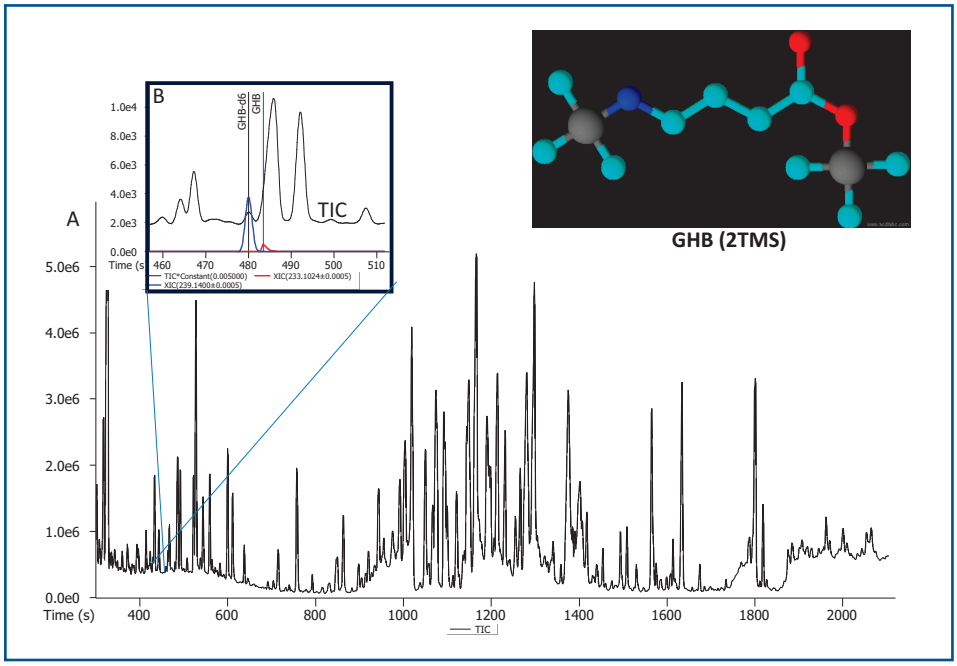

Figure 1. A) Total Ion Chromatogram (TIC) of a Hair Sample Showing GHB (2TMS). The B) Inset demonstrating the ability of accurate mass measurements to eliminate matrix interferences.

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2. Experimental

Hair samples were obtained from two volunteers (A and B) and washed three times with deionized water with sonication for 5 minutes followed by triplicate washings with dichloromethane with sonication for 5 minutes. The samples were then dried between filter paper overnight and split into two sets: 1) Bead Ruptor set (A1, A2; B1, B2) and 2) Cut set (A3, A4; B3, B4). The Bead Ruptor set was homogenized using a Biotage Bead Ruptor 24. Set 2 was cut into 1-3 mm segments using scissors. Each sample (20 mg each), as well as a blank and 5 calibrators (0.15-5.0 ng/mg GHB), were placed in microcentrifuge tubes and 1mL of methanol 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 N2 gas. Derivatization was carried out by mixing the dry samples with 100 µL of BSTFA and heating for 1 hour at a temperature of 60°C. The resulting products were transferred to 2 mL GC vials for GC-HRT analysis.

Table 1. GC-High Resolution TOFMS Conditions.

Gas Chromatograph	**Agilent 7890 with 7693A Autosampler**
Injection	1 µL, Pulsed Splitless @ 250°C
Carrier Gas, Flow	He @ 1.5 mL/min, Constant Flow
Column	Rxi-5MS, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Temp. Program	70°C (1 min) to 320°C @ 8°C/min (10 min)
Transfer Line	300°C
Mass Spectrometer	**LECO Pegasus GC-HRT (R = 25,000)**
Ion Source Temp.	250°C
Acquisition Mode	High Resolution, R = 25,000 (FWHM)
Ionization Mode, Energy	EI, 70 eV
Mass Range (m/z)	45-510
Acquisition Rate	10 spectra/s

3. Results and Discussion

ChromaTOF-HRT brand software was used to process data for qualitative (HRD, comprehensive processing) and quantitative analysis (Target Analyte Finding or TAF, quantitative processing). TAF of GC-HRT data was used to determine the concentration of GHB in each sample (Figure 2). Using the TAF feature of ChromaTOF-HRT is a convenient method for retrospective analysis of data and involves the addition of analytes, internal standards, and the corresponding accurate mass ion retention time windows with mass tolerances (Da, mDa or ppm) to target tables. It is ideal for the processing of large sample sets and/or for trace analysis. The calibration curve for GHB (internal standard = GHB-d6) was constructed using the quantitation and reference ions m/z = 233.1024 and 239.1400 (Figures 3,4). Application of the curve to samples A and B using ChromaTOF-HRT processing resulted in the GHB concentrations listed in Table 2. Concentrations for the A and B "Bead" samples were greater than the "Cut" samples. In addition, there was more GHB in volunteer B as compared to volunteer A's sample hair.

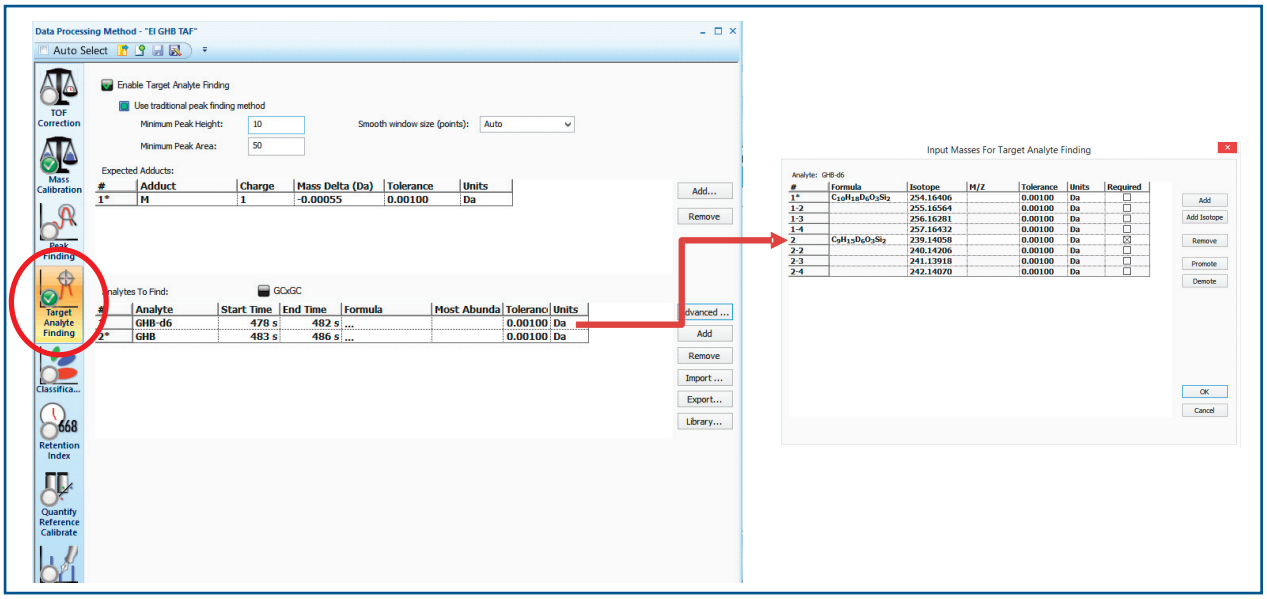

Figure 2: Target Analyte Finding Method for Quantitation of GHB in Hair.

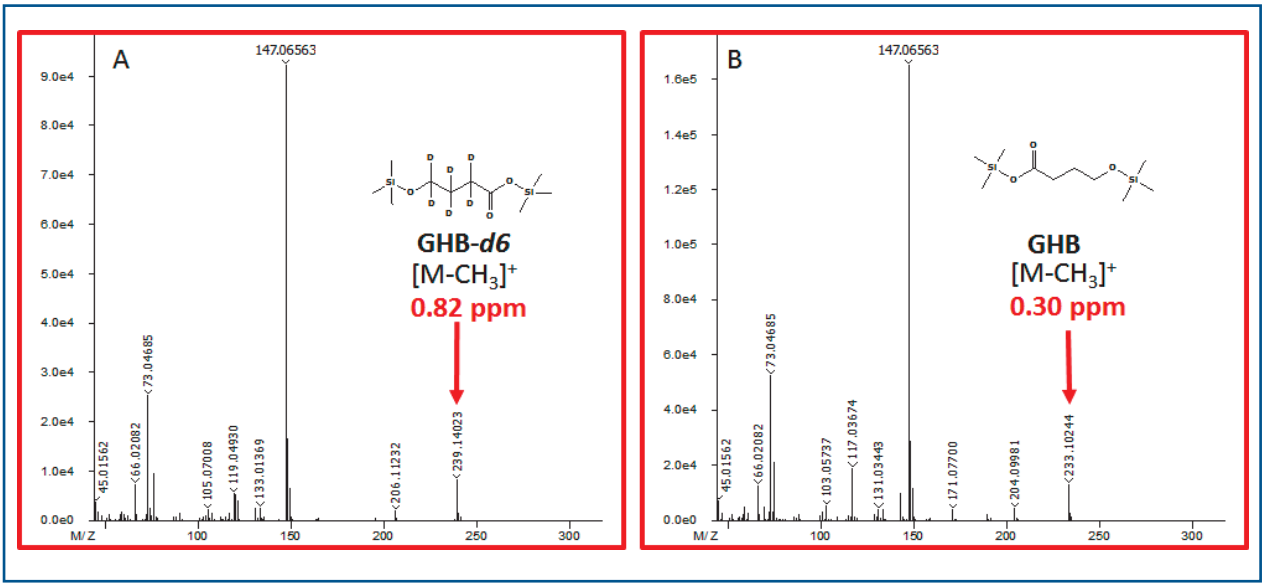

Figure 3: Peak True Mass Spectral Data for A) GHB-d6 and B) GHB.

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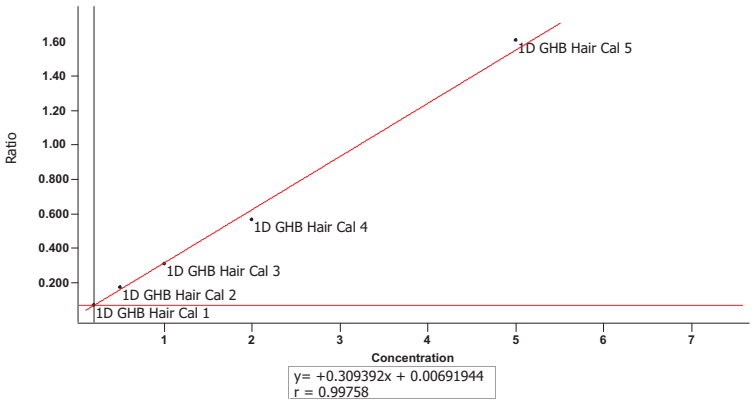

Standard	Area	Calc. Conc.	Cert. Conc.	Weighting
1D GHB Hair Cal 1	12048	0.21	0.20	5
1D GHB Hair Cal 2	28695	0.53	0.50	2
1D GHB Hair Cal 3	60094	0.97	1.00	1
1D GHB Hair Cal 4	95763	1.81	2.00	0.5
1D GHB Hair Cal 5	257491	5.19	5.00	0.2

Table 2: GHB Concentrations (ng/mg) in Samples A and B.

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

Figure 4 : GHB Calibration Curve (A) and Tabular Information (B). Internal Standard = GHB-d6.

Hair is a complex mixture of chemically diverse compounds (Figure 1). Comprehensive data processing (Peak Find) of GC-HRT data resulted in identification of a wide variety of compounds including acids, amino acids, diacids, fatty acids, esters, amides and monoacylglycerides (Table 3). High quality spectral data was searched against large, well-established databases (e.g., NIST, Wiley) to identify metabolites in hair. Compound identification was confirmed by leveraging accurate mass ions in the Peak True (Deconvoluted) mass spectral data as shown for serine (3TMS) in Figure 5 (spectral similarity = 938/1000). Mass accuracy values for serine's $[M\text{-}C_4H_9O_2Si]^+$ and $[M\text{-}C_4H_{11}OSi]^+$ were -0.17 and -0.40 ppm respectively. Mass accuracy values for the representative set of compounds in Table 1 ranged from -1.59 to 0.96 ppm (Ave. |ppm| = 0.56).

Table 3: Representative Compounds in Hair.

Name	Formula	R.T. (s)	Area	Similarity	Ion	MA (ppm)
Urea (2TMS)	$C_7H_{20}N_2OSi_2$	486	12127269	928	$M^{\bullet+}$	-0.24
Benzoic Acid (TMS)	$C_{10}H_{14}O_2Si$	492	5448582	912	$M^{\bullet+}$	-0.32
Methylmalonic Acid (2TMS)	$C_{10}H_{22}O_4Si_2$	559	10518373	950	$M^{\bullet+}$	0.67
Serine (3TMS)	$C_{12}H_{31}NO_3Si_3$	611	4989159	938	$[M\text{-}C_4H_{11}OSi]^+$	-0.40
4-hydroxybenzaldehyde (TMS)	$C_{10}H_{14}O_2Si$	614	125596	846	$M^{\bullet+}$	0.96
5-*oxo* -Proline (2TMS)	$C_{11}H_{23}NO_3Si_2$	757	20156041	936	$M^{\bullet+}$	0.32
Phenylalanine (2TMS)	$C_{15}H_{27}NO_2Si_2$	849	3862385	883	$[M\text{-}C_7H_7]^+$	-0.03
Myristic Acid (TMS)	$C_{17}H_{36}O_2Si$	976	3647162	841	$[M\text{-}CH_3]^+$	-1.59
Uric Acid (4TMS)	$C_{17}H_{36}N_4O_3Si_4$	1222	277571	789	$M^{\bullet+}$	0.63
9Z,12Z-Octadecadienoic Acid (TMS)	$C_{21}H_{40}O_2Si$	1266	3539559	784	$M^{\bullet+}$	-0.66
Stearic Acid (TMS)	$C_{21}H_{44}O_2Si$	1298	32507255	904	$M^{\bullet+}$	-0.83
9-Octadecenamide	$C_{18}H_{35}NO$	1375	26423908	844	$M^{\bullet+}$	-0.65
1-Monomyristin (2TMS)	$C_{23}H_{50}O_4Si_2$	1398	2094427	828	$[M\text{-}C_4H_{11}OSi]^+$	-0.03

Ave. |ppm| = 0.56

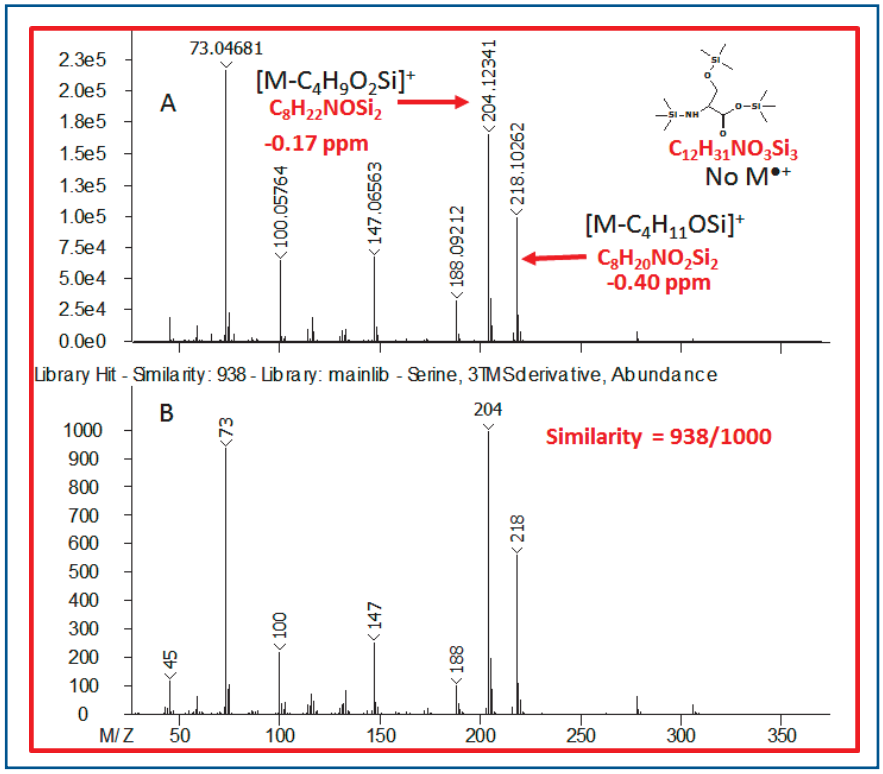

Figure 5: A) Peak True and B) NIST Library Mass Spectral Data for Serine (3TMS).

4. Conclusion

Hair extracts consisted of a complicated mixture of compounds transferred internally from the blood or externally from sources such as conditioner, sun screen, or other commercial products. Greater quantities of GHB were extracted using the "Bead Ruptor" rather than "Cut" sample preparation methods. In addition, more GHB was found in the hair of volunteer B. The enhanced resolving power of the GC-HRT minimized interferences and facilitated accurate quantitation of GHB in hair using Target Analyte Finding processing, while the comprehensive nature of TOF data acquisition with HRD allows laboratories to detect and identify other potentially important analytes in a single data file

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