# Extraction, Analysis and Quantization of Endogenous Level of GHB from Human Hair

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## Abstract

*Hair is a physical evidence to describe criminals. Hair plays a multi-disciplinary role, role of forensic chemist to identify drug from hair and a forensic biologist can take DNA. It can give information of drug history, identification of human or animal, mode of hair removed from skin, and DNA profile of person may be victim or criminal. According to Baumgartner et al., to collect hair sample we do not need any special equipment as well as for storage. Presence of drugs in urine and fecal analysis zero to 3 or 4 days only but for hair it is months to years. Drugs analysis of hair became very common in Forensic laboratory because a long enough hair can present long term history of drugs taking by individual. Tt is acknowledged that in common, average growth of hair in every person is 1 cm per month. So segment of hairs sample can give information of drugs taken during months of calendar year. According toKintz et al, "detection of gamma-hydroxy butyrate (GHB) in hair following an alleged drug-facilitated sexual assault (DFSA)." There are a number of cases reported which are related to Gamma Hydroxybutyrate (GHB) abuse. It is a club or date rape drug, used for sexual and other abuse. The aim of this research will be to describe what is the best technique that can be used for the analysis of hair to get endogenous level of GHB in hair? Decontamination of hair done by washing with 5 ml of methylene chloride twice at room temperature. To predict correct interpretation washing is required. To improve quality of results we operate chromatography –mass spectroscopy (GC-MS) test. It is difficult to make cross relativity between qualitative and quantitative data for immunological test to give conformity results but GC-MS present very analogous data. Kronstrand et al., These are some different standard concentrations, which were used in this laboratory work for analysis. In our experiment, we also took three different samples weighing 20mg each for analysis. Data of GC-MS results, gives pick area for the quantization ions 117(m/z), 147 (m/z), 233 (m/z), internal standard 145 (m/z) as well as the standard concentrations and our three samples. From these, we took ion 233(m/z) as Q, and together with the internal standard 145 (m/z), the table have prepared for peak area ratio of the various concentrations from by the formula 233(m/z)/IS. Ions selected for Single Ion Monitoring (SIM) of GHB were 233, 204 and 117 m/z (graph 1.0), for result of this experiment this are $C_1=117$, $C_2=147$ and $Q = 233$. The 5-points calibration curves were linear over the concentration range evaluated. The resultant linear regression equation extrapolated from graph 1.1 is given as (y = 0.0036x + 0.0033), where 0.0036 represents the gradient and 0.0033 being the intercept. It also has a measure of precision or the coefficient of goodness of how well experimental data is spread on the line as ($R^2$). From the literature, previous studies by were excellent ($R^2=0.9947$ for this experiment). Limit of quantisation (LOQ) was identified as the lowest calibrator point is102.5 ng/mg.Role of drug analysis from hair sample to give information of drug history and quantity of used. GC-MS technique which have big contribution now and in further development of forensic science because their aspects are endless.*

**Key words:** Quantization of endogenous level of GHB, Toxicology, Toxicologist to identify drug from hair, Drugs analysis of hair, Gamma-Hydroxy Butyrate (GHB), Club or Date Rape Drug, Chromatography –Mass Spectroscopy (GC-MS), Limit of Quantisation (LOQ), Concentration of drugs in hair.

## Introduction

As according to Locard physical evidence distorted among people and during the time of crime. This physical evidence is analyzed in criminal laboratory to find out criminals. Hair is also a physical evidence to describe criminals[1] (P-208 and 233). Person who is responsible to visit a crime scene may be police or forensic expert, It is very important to collect hair sample carefully because it plays a multi-disciplinary role, role of forensic chemist to identify drug from hair and a forensic biologist can take DNA. Communication between everyonecan lead valuable information for crime. It is more important because it can give information of drug history, identification of human or animal, mode of hair removed from skin, and DNA profile of person may be victim or criminal[2]. There are a number of cases reported which are related to Gamma Hydroxybutyrate (GHB) abuse, it was 110 in 1998, 150 in 1999, 120 in 2000, 119 in 2001, 100 in 2002, 66 in 2003, 84 in 2004, and 62 in 2005. All these data belong to only Texas Poison Control Centres and also more number of abuses in other cities.

It is a club or date rape drug, used for sexual and other abuse. This essay will first demonstrate the basic importance of hair and why hair is important for an aspect to drug analysis with a brief case history? Then it will describe briefly some methodology; which can be applied on hair evidence in the laboratory work to get better results. Then we will take a critical evaluation of results of this experiment. Finely this essay will discuss brief results of experiments followed by conclusion. The aim of this essay will be to describe what is the best technique that can be used for the analysis of hair to get endogenous level of GHB in hair?

**Background and Introduction:** Drugs analysis of hair became very common in Forensic laboratory because a long enough hair can present long term history of drugs taking by individual but not exact date of drug taken. In addition it is acknowledged that in common, average growth of hair in every person is 1 cm per month. So segment of hairs sample can give information of drugs taken during months of calendar year. According to Kintz et al[3], "detection of gamma-hydroxy butyrate (GHB) in hair following an alleged drug-facilitated sexual assault (DFSA)." 19 year old female have been sexual assault after drinking. After the crime and one month later hair sample taken from her. Both hair sample cut in segments during examinations. Hair sample which is taken after 1 month of crime shows prances of GHB. It is important that there shouldn't be use of any hair cosmetic treatments or dye to prove GHB in hair sample during I month. Environmental factor also effect existence of drugs in hairs like being always in smoky room gives negative result for drugs but it gives positive result if hairs sample washed in buffer solutions

followed by acetone to make sure drug is examine from inner layer of portion (cortex) of hair not from contamination area or outer area of hairs (cuticle)[2].

According to Baumgartner and et al.[4], to collect hair sample we do not need any special equipment as well as for storage. Time line prove storage difficulty in a graph (figure-1) which describe presence of drugs in urinalysis and fecal shavings analysis zero to 3 or 4 days only but for hair it is months to years[5].

## Methodology

Vertex posterior is a best place (back of the head) to collect hair sample compared to other area of head because hair is less subjected to age and sex, less changeability in hair growth rate, number of hairs in growth place is more regular. Widespread population has physiological concentration of GHB in their hair. Toxicologist can analyze change in concentration of GHB level but level of GHB is different at root of hair[6]. After collecting hair, packing is important for further examination, and then it involved visual characteristics of hair like length, shape, size, color and degree of straightness or rigidity after visual characteristics hair should be observed on low power micro scope. If root is present with hair so root should be cut from the shaft of hair for DNA source otherwise it will be difficult to get DNA in future but not impossible. According to Birngruber et al, 2009; study of hair has shown, intra-individual difference within in the single hair because of cosmetic treatments on hair. Robertson said, difference between human and animal hair can be seen with different parameters and cross-sections on hair[2].
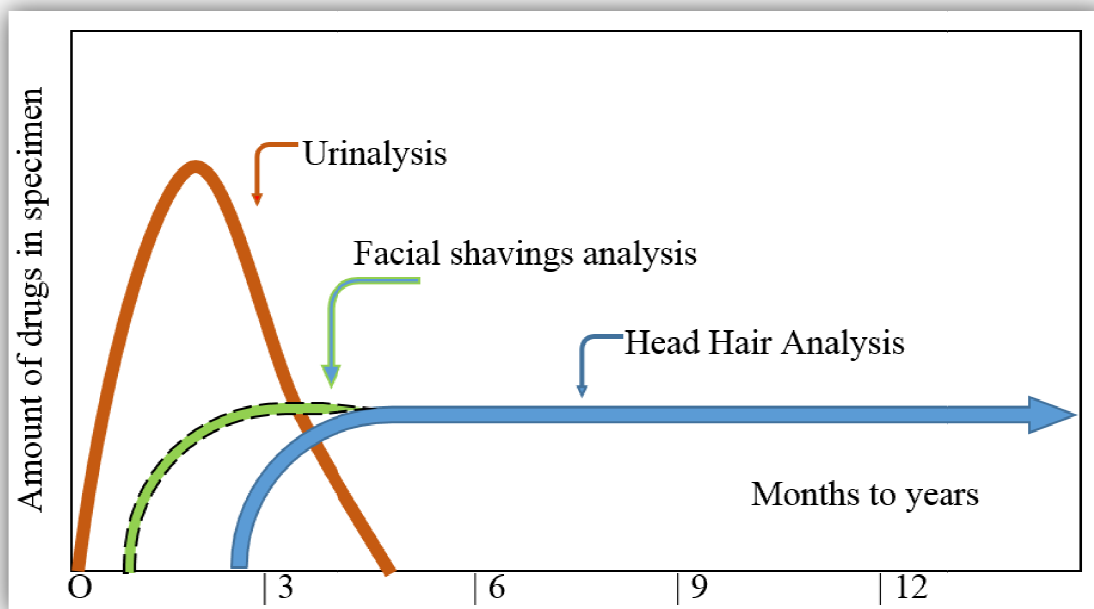


**Figure-1**
**Drug information from hair[7]**

There are some measurements involved to confirm presence of drug in hair and all of this experiment can give satisfactory and suitable result. Washing of hair sample and extraction of drugs from above have Varity of process, due to this, it is very difficult to compare all the results. "Several papers have evaluated different procedure for removing drugs such as cocaine and heroin metabolites from the hair matrix". This method says, at evaluate temperature with strong acid or base. According to Baumgartner and Hill[4] projected universal extraction for any substance as an enzymatic extraction at natural PH. Method of extraction and detection are very similar between urine, blood or plasma after taking drug out from hair sample. To improve conditions of hair sample, it is good advantages to collect hair sample before autopsy so we can avoid contamination of drug from hair[6]. According toKintz at al[3], decontamination of hair done by washing with 5 ml of methylene chloride twice at room temperature[7]. In 1 to 3 days duration of coma after acute intoxication of blood and urine can give negative result due to elimination and also cut hair may give negative test also. After collection of hair it should be wrapped in aluminium foil to preserve. Segmentation of hair is very important in date-rape cases and alleged criminal poisoning, which can lead to prove particular drug and time duration when it has taken[5].

In 1979 Radioimmunoassay of hair (RIAH) described by Baumgartner et al[4] for detection of heroin/morphine then result of experiments shows hair analysis and urine analysis are complimentary technique to each other because qualitative information and recent drug use shown by urine analysis in other hand quantitative information's given by hair analysis as a long tram history of drugs using by individual[5]. To predict correct interpretation washing is required. Probably main contamination of drugs in hair comes from sweat and it is panic with curly hair. Radioimmunoassay and enzyme – linked immunosorbent assay (ELISA) are quick and economical for screening test. Importance of using both of these methods can make easy to compare result to conform the best result. In addition to improve quality of results we can operate liquid chromatography –mass spectroscopy (GC-MS) test. It is difficult to make cross relativity between qualitative and quantitative data for immunological test to give conformity results but GC-MS present very analogous data. Kronstrand et al., using LC-MS/MS they describe screening method in multiple reaction mode. GC-MS is a conquered technique from 15 years but at present LC-MS/MS rising as a legal confirmation technique[6].

## Results and Discussion

These are some different standard concentrations in a table-1, which were used in this laboratory work for analysis. In our experiment, we also took three different samples weighing 20mg each for analysis. Data of GC-MS results, gives pick area for the quantization ions 117(m/z), 147 (m/z), 233 (m/z), internal standard 145 (m/z) as well as the standard concentrations and our three samples. From these, we took ion 233(m/z) as Q, and together with the internal standard 145 (m/z), the table below was prepared for peak area ratio of the various concentrations from by the formula 233(m/z)/IS.

**Discussion:** Previously describedIons selected for Single Ion Monitoring (SIM) of GHB were 233, 204 and 117 m/z (figure-4), for result of this experiment this are $C_1=117$, $C_2=147$ and Q = 233. The 5-points calibration curves were linear over the concentration range evaluated. The resultant linear regression equation extrapolated from figure-5 is given as (y = 0.0036x + 0.0033), where 0.0036 represents the gradient and 0.0033 being the intercept. It also has a measure of precision or the coefficient of goodness of how well experimental data is spread on the line as ($R^2$). From the literature, previous studies by were excellent ($R^2$=0.9947 for this experiment). Limit of quantisation (LOQ) was identified as the lowest calibrator point is[8] 102.5 ng/mg.

**Table-1**
**show the experimental Data of GC-MS results, with peak area ratio (source, own data from experiment)**

| Concentration | Peak Area (m/z 117) C1 | Peak Area (m/z 147) C2 | Peak Area (m/z 233) Q | Internal Standard (m/z 145) IS | Q/IS | Concentration |
|---|---|---|---|---|---|---|
| Standard 1 µg/ml | 88509 | 377957 | 53144 | 16370406 | 0.003246 | 1 |
| 5 µg/ml | 611803 | 2467560 | 318925 | 16677923 | 0.019123 | 5 |
| 10 µg/ml | 1272690 | 5525672 | 688517 | 17064418 | 0.040348 | 10 |
| 20 µg/ml | 2678139 | 8072404 | 1394358 | 16379056 | 0.085131 | 20 |
| 50 µg/ml | 5516174 | 10836737 | 3044781 | 16697294 | 0.182352 | 50 |
| Sample A1 | 447074 | 3972486 | 241806 | 9131451 | 0.026481 | |
| A2 | 52776 | 154805 | 30297 | 6660228 | 0.004549 | |
| A3 | 14989 | 69639 | 9116 | 8996688 | 0.001013 | |
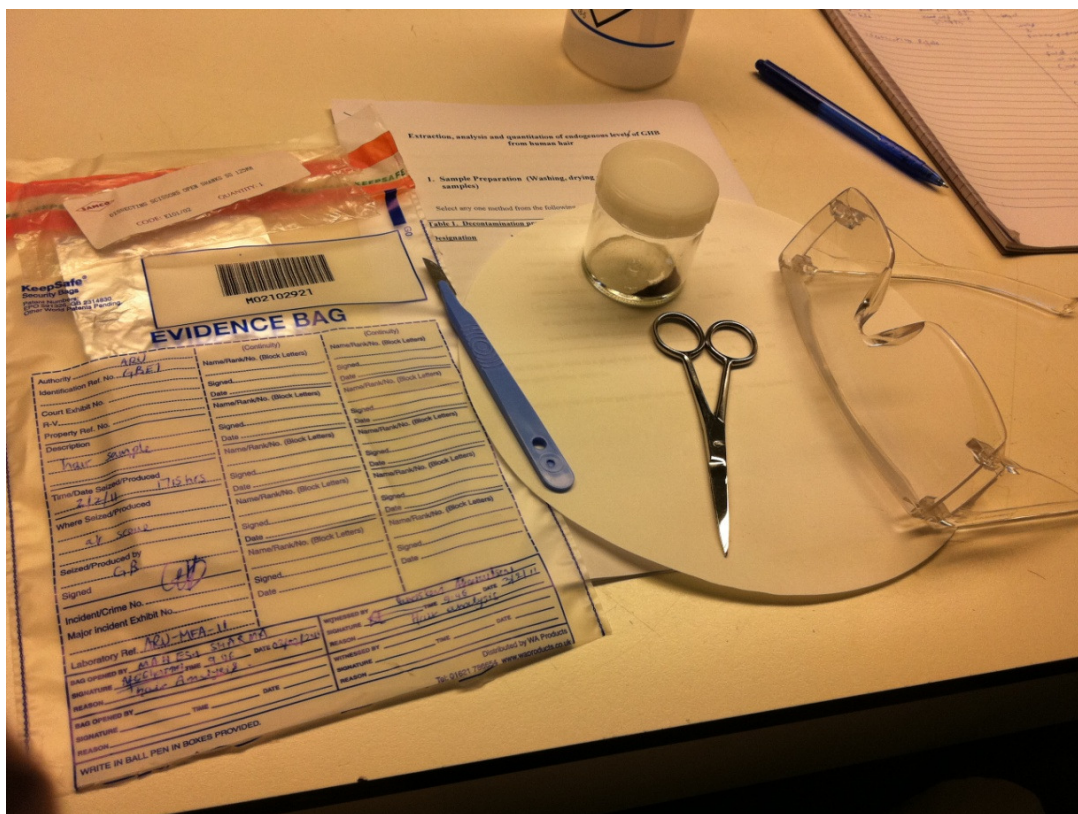
**Figure-2**
**Evidence bag with aluminum foil to keep hair and some requirments.Source: Own collections**



**Figure-3**
**Samples and different concentration preparation for GHB analysis.Source: Own collections**
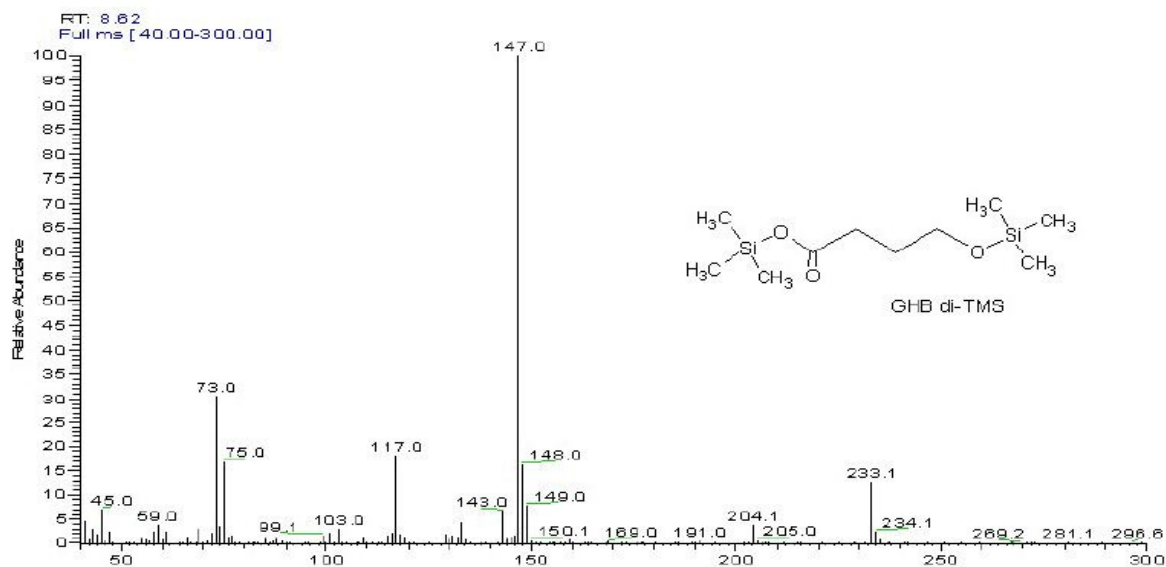
**Figure-4**
**Mass spectra of the trimethylsilyl derivative of GHB (GHB-di-TMS) and Ions selected for Single Ion Monitoring (SIM) of GHB di-TMS are m/z 233, 204 and 117 Source[8]**
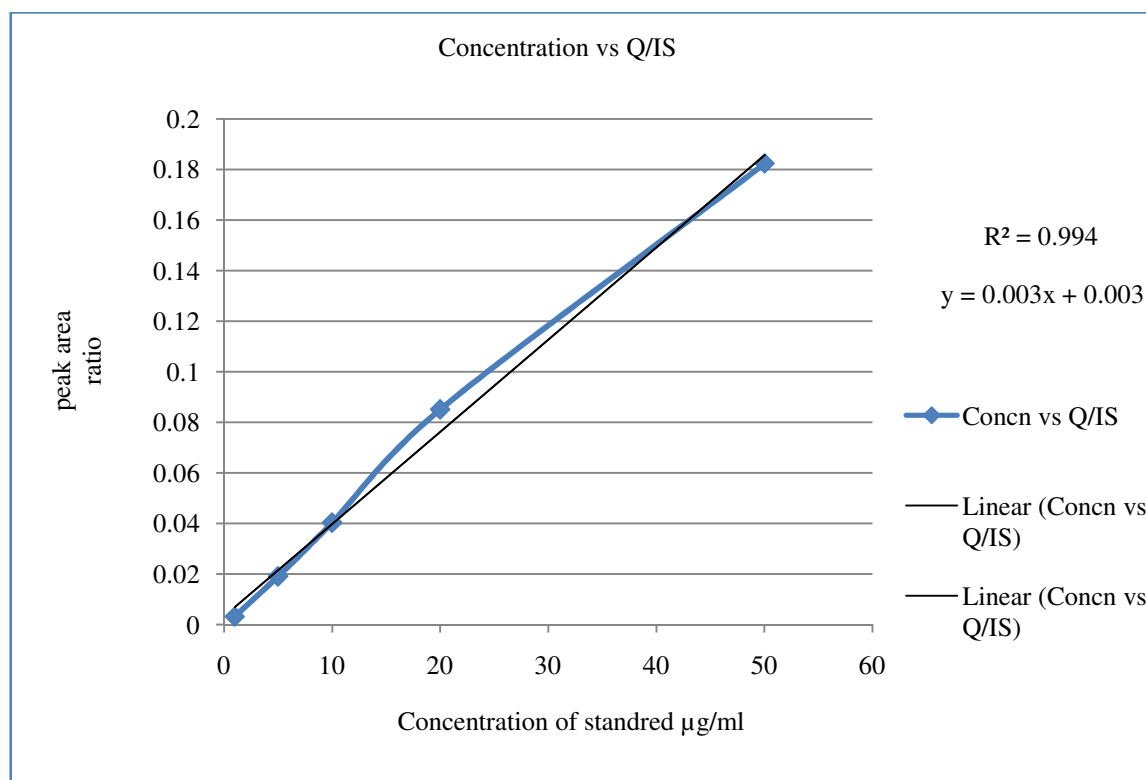


**Figure-5**
**Calibration graph based results of this experiment**

## Conclusion

The aim of this essay was to evaluate role of drug analysis from hair sample to give information of drug history and quantity of used. To support this question first some basic information on hair, GHB abused data was described. In addition we looked at 19 year old female case shows presence of GHB in hair sample taken after 1 month not in 4 days. Also we looked at some important points to get better results and according to level 4 student comparisons with other technique. In addition GC-MS

technique which have big contribution now and in further development of forensic science because their aspects are endless.

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