

**FORENSIC TOXICOLOGY LABORATORY  
OFFICE OF CHIEF MEDICAL EXAMINER  
CITY OF NEW YORK**

**GAMMA-HYDROXYBUTYRATE (GHB)  
by  
SOLID PHASE EXTRACTION and  
GAS CHROMATOGRAPHY/MASS SPECTROMETRY  
(Selective Ion Monitoring)**

**PRINCIPLE**

Gamma-hydroxybutyrate (GHB) is an endogenous metabolite of gamma-aminobutyric acid (GABA) and is believed to be a cerebral neurotransmitter. It has been utilized clinically as an anesthetic and hypnotic since the 1960's. Xyrem® (sodium oxybate) oral solution is currently as an orphan drug being researched to treat the combination of sleep disorders known as narcolepsy/cataplexy. It has also been used illicitly, sold in health food stores for weight control and gymnasiums as a steroid alternative for body building. Currently it is considered to be one the most commonly used "date rape" drugs. Illicit GHB use usually involves oral doses of the powder dissolved in water. GHB is currently a Schedule II drug.

Many methods analyze GHB by converting to the butyrolactone (GBL). Other methods derivatized by silylation. The current method employs the chemical derivitization of GHB. GHB is extracted from biological specimens by solid phase extraction. In solid phase extraction the drugs are temporarily bound to a sorbent in the solid phase cartridge as the prepared sample is poured through the column. The column is washed to remove interfering compounds, followed by elution of the drugs desired from the column. The eluted drug is silylated and injected on GC/MS for SIM.

**SAFETY**

The handling of all biological specimens and reagents is performed within the guidelines which are detailed in the Safety and Health manual.

**SPECIMEN PREPARATION**

The procedure is routinely applied to the following biological specimens and their aliquots unless otherwise specified:

Blood	200 µL of the undiluted specimen
Urine	200 µL for qualitative identification
Gastric Contents	200 µL of a 1:10 dilution

## REAGENTS AND MATERIALS

All chemicals should be analytical reagent grade or better.

1. **Deionized water** (distilled can be substituted)
2. **Methanol** (Fisher Scientific - ACS certified, or equivalent)
3. **Acetone** (Fisher Scientific - ACS certified, or equivalent)
4. **Ammonium Hydroxide, NH<sub>4</sub>OH** (Fisher Scientific - ACS certified, or equivalent)

**Note:** Ammonium hydroxide will break down to ammonia and water and the ammonia will evaporate if the container is not kept closed. This will cause the pH decrease, making the reagent unsuitable for solid phase extraction. Use small lots of working solution (3 mL of ammonium hydroxide in a 6 mL crimp top vial. Use once then discard the remaining), open a fresh bottle daily. Always keep ammonium hydroxide refrigerated.

5. **100 mM phosphate buffer (pH 6.0)**

Dissolve 3.40 g Na<sub>2</sub>HPO<sub>4</sub> and 24.28 g NaH<sub>2</sub>PO<sub>4</sub> H<sub>2</sub>O in 1000 mL DI H<sub>2</sub>O.

Dilute to 2000 mL using DI H<sub>2</sub>O. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH). Store at 5 °C in glass.

Stability: 1 month. Inspect each day of use for contamination.

6. **Eluting solvent**

**CH<sub>3</sub>OH/NH<sub>4</sub>OH (99/1)**. Prepare fresh each day of use.

7. **BSTFA** with 1% TMCS (N,O-bis(trimethyl)trifluoroacetamide with 1% Trimethylchlorosilane)
8. **GHB-d<sub>6</sub>** (internal standard), Cerilliant or equivalent, 1.0 mg/mL in 1mL methanol, 99% pure (or equivalent).
9. **GHB-d<sub>6</sub> working internal standard solution 100 mg/L**
  - a. Pipette 1.0 mL of 1.0 mg/mL GHB-D<sub>6</sub> to a 10 mL volumetric flask.
  - b. Q.S. to 10 mL with H<sub>2</sub>O to result in a 10.0 mg/L solution.
  - c. Transfer into properly labeled container (This should include identity, concentration, solvent, lot number, date prepared, and initials of analyst). Store at 2- 4° C.
10. **GHB**, Cerilliant or equivalent, 1.0 mg/mL in 1 mL methanol, 99% pure, or equivalent.
11. **GHB calibrator and control solution, 200 mg/L in H<sub>2</sub>O**
  - a. Pipette 2 mL of 1.0 mg/mL GHB to a 10 mL volumetric flask.
  - b. Q.S. to 10 mL with H<sub>2</sub>O.

- c. Transfer into properly labeled container (include identity, concentration, solvent, lot number, date prepared, and initials of analyst). Store at 2-4°C.

Repeat the above procedure to produce a 200 mg/L control solution, using a different lot of GHB if practical.

## 12. Negative blood

Calf or sheep blood obtained from outside source or equivalent. Validated as negative by in-house analysis. Store frozen (-10 °C or lower).

13. **Solid Phase Columns:** Part #ZSGHB020 (Worldwide Monitoring, from United Chemical Technologies)

## 14. Gas Chromatography Column:

Restek RTX-50 (Crossbond 100% methyl phenyl polysiloxane). 30M x 0.25 mm ID x 0.25 µm DF, max temperature 320 °C. Model # 10523

## PROCEDURE

1. Aliquot 200 µL of sample or validated negative matrix into each 16 x 125 mm test tube labeled as to the contents (sample, calibrator, control, or blank).
2. Add appropriate amounts of standard solutions to negative matrix tubes as follows:

Five positive calibrators and one blank should be run with each batch of samples. Positive calibrators are prepared in the following concentrations:

- 5 mg/L - add 5 µL of 200 mg/L working calibrator solution
- 10 mg/L - add 10 µL of 200 mg/L working calibrator solution
- 25 mg/L - add 25 µL of 200 mg/L working calibrator solution
- 50 mg/L - add 50 µL of 200 mg/L working calibrator solution
- 100 mg/L - add 100 µL of 200 mg/L working calibrator solution

**Note:** Deionized water is used as the negative matrix for urine and gastric specimens.

Two controls are included in the run. A positive control of 7.5 mg/L is prepared as follows: add 7.5 µL of 200 mg/L control working solution to the negative matrix. A second positive control of 50 mg/L is prepared as follows: add 50 µL of 200 mg/L control working solution to the negative matrix.

3. Add 50 µL of 100 mg/L working internal standard solution to all tubes. The concentration of the internal standard in each sample is 25 mg/L.
4. Add 1 mL of acetone. Mix by Vortex for 15 seconds. Centrifuge at ≈3000 rpm for 10 minutes. Transfer acetone layer to a properly labeled culture tube.
5. Evaporate acetone at 80 °C with nitrogen.
6. Reconstitute the dried extract with 200 µL of 0.1 phosphate buffer pH 6.0. Mix by Vortex 15 seconds.

7. Condition extraction columns (use ZSGHB020). This can be done while samples spin down. Columns must be re-conditioned if they dry out before a sample is applied.

Pour 3 mL CH<sub>3</sub>OH onto column; by gravity.

Pour 3 mL DI H<sub>2</sub>O onto column; by gravity.

Pour 3 mL 100 mM phosphate buffer (pH 6.0) onto column; by gravity.

**Note:** Do not allow the column sorbent to dry.

8. Apply sample. The sample should flow through the column no faster than 1 to 2 mL/minute. Make sure pellet remains in test tube. Apply vacuum (up to 3 inches Hg) if necessary to achieve the desired flow. Dry 15 minutes under high vacuum.

9. Elute drug

Place clean labeled 10 mL conical test tubes into rack.

Add 1.0 mL CH<sub>3</sub>OH/NH<sub>4</sub>OH (99/1) to column.

Collect extract.

**Note:** Prepare elution solvent daily.

10. Concentrate

Evaporate eluate to dryness at 70°C using a stream of nitrogen.

**Note:** This usually takes approximately 30 minutes.

11. Derivatize

Add 100 µL ethyl acetate and 100 µL of BSTFA with 1% TMCS. Cap test tube. Mix. Vortex. Heat at 70 °C for 30 minutes.

12. Label autosampler vials indicating aliquot and toxicology number (ex: 2-YY-xxxx), specimen type, dilution, analyst and date. Immediately seal each vial with an aluminum seal using a crimper to avoid possible contamination from other samples. Samples may also be transferred into screw cap vials and capped immediately. Physically check that the crimped seal is tight by attempting to rotate the seal. Crimp until tight, using a new seal if necessary. Do not wait until all transfers have been made to seal the vials.

13. Transfer sealed vials to GCMS section for analysis.

## INSTRUMENTATION

Agilent MSD 6890 GC with 5973 Mass Spectrometer, Agilent 7683 Autosampler and Agilent Chemstation with current revision of software. The method name for this assay is MSNGHB.M, where N = instrument number.

The following ions are monitored:

GHB-D<sub>6</sub> diTMS : 239, 240, 241

GHB di TMS : 233, 234, 235

## TOP LEVEL PARAMETERS

Method Information For: C:\MSDCHEM\1\METHODS\MSNGHB.M

Method Sections To Run:

- Save Copy of Method With Data
- Instrument Control Pre-Run Cmd/Macro =
- Data Analysis Pre-Run Cmd/Macro =
- Data Acquisition
- Data Analysis
- Instrument Control Pre-Run Cmd/Macro =
- Data Analysis Pre-Run Cmd/Macro =

Method Comments:

This is the GHB method.

**END OF TOP LEVEL PARAMETERS**

## INSTRUMENT CONTROL PARAMETERS

Sample Inlet	GC
Injection Source	GC ALS
Mass Spectrometer	Enabled

## HP6890 GC METHOD

### OVEN

Initial temp	70 °C (On)	Maximum temp	320 °C
Initial time	1.00 min	Equilibration time	0.50 min

<i>Ramps</i>			
#	Rate	Final temp	Final time
1	15.00	100	0.00
2	20.00	175	0.00
3	35.00	280	3.00
4	0.00(Off)		
Post temp	290 °C		
Post time	3.00 min		
Run time	12.75 min		

<i>Front Inlet (Unknown)</i>	<i>Back Inlet ()</i>
Mode	Split
Initial temp	250 °C (On)
Pressure	8.80 psi (On)
Split ratio	2.6:1
Split flow	2.6 mL/min

Total flow	5.4 mL/min	
Gas saver	On	
Saver flow	20.0 mL/min	
Saver time	3.00 min	
Gas type	Helium	

Column 1		Column 2
Capillary Column		(not installed)
Model Number	Restek RTX 50	
Max temperature	320 °C	
Nominal length	30.0 m	
Nominal diameter	250.00 µm	
Nominal film thickness	0.25 µm	
Mode	constant flow	
Initial flow	1.0 mL/min	
Nominal init pressure	8.81 psi	
Average velocity	37 cm/sec	
Inlet	Front Inlet	
Outlet	MSD	
Outlet pressure	vacuum	

FRONT DETECTOR (NO DET)	BACK DETECTOR (NO DET)
COLUMN COMP 1	COLUMN COMP 2
(No Detectors Installed)	(No Detectors Installed)
SIGNAL 1	SIGNAL 2
Data rate: 20 Hz	Data rate: 20 Hz
Type: test plot	Type: test plot
Save Data: Off	Save Data: Off
Zero: 0.0 (Off)	Zero: 0.0 (Off)
Range: 0	Range: 0
Fast Peaks: Off	Fast Peaks: Off
Attenuation: 0	Attenuation: 0

Thermal Aux 2	
Use: MSD Transfer Line Heater	
Description	
Initial temp	280 °C (On)
Initial time	0.00 min
# Rate Final temp Final time	
1 0.0(Off)	

Post Run	
Post Time	3.00 min
Oven Temperature	290 °C
Column 1 Flow	1.0 mL/min

<i>Time Table</i>		
<b>Time</b>	<b>Specifier</b>	<b>Parameter &amp; Setpoint</b>
7673 Injector		
<i>Front Injector:</i>		
Sample Washes	1	
Sample Pumps	2	
Injection Volume	3.0 microliters	
Syringe Size	10.0 microliters	
PostInj Solvent A Washes	3	
PostInj Solvent B Washes	3	
Viscosity Delay	0 seconds	
Plunger Speed	Fast	
Preinjection Dwell	0.00 minutes	
Postinjection Dwell	0.00 minutes	
<i>Back Injector:</i>		
No parameters specified		

#### MS ACQUISITION PARAMETERS

<i>General Information</i>	
Tune File	Atune.u
Acquisition Mode	SIM
<i>MS Information</i>	
Solvent Delay	4.0 min
EMV Mode	Gain Factor
Gain Factor	1
Resulting EM Voltage	800

[Sim Parameters]

<i>Group 1</i>			
Group ID	1		
Resolution	High		
Plot 1 Ion	233.0		
Plot 2 Ion	241.0		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(233.0, 75)	(234.0, 100)	(235.0, 125)
	(239.0, 75)	(240.0, 100)	(241.0, 125)

[MSZones]

MS Quad: 150 °C maximum 200 °C  
MS Source: 230 °C maximum 250 °C

## END OF MS ACQUISITION PARAMETERS

## END OF INSTRUMENT CONTROL PARAMETERS

### INSTRUMENT SETUP

An acceptable autotune must be obtained prior to batch analysis. Refer to the SOP entitled "GCMS Tuning" for instructions. All autosampler syringe wash vials are filled with ethyl acetate.

Prepare a sequence using the following steps.

When Chemstation is opened, the **Openlab ECM Login** screen appears, enter the instrument name (ms3, ms4, etc.) as appropriate for username and the current password. Verify that Account field says Production and Domain field says Built-In. If Chemstation is already running, it may be necessary to log out andre-login. Using the Chemstation software, at the top Method and Run toolbar under **ECM**, select **Login to ECM**. Follow the instructions above to log on.

1. On the Method and Run toolbar, under **Sequence**, select **Load Sequence**. Select **default.s**. Click on Select.
2. In the Method and Run toolbar, under **Sequence**, select **Edit Sequence**. At the top of the screen under Data Path, click on Browse. Under **Select Data Path**, click on the **msdchem** folder. Then click **1** and then click on the **Data** folder to highlight it. In lower left of dialog box, select **Make New Folder**. A folder with the name New Folder is created under DATA. Right click on **New Folder** and **Rename** or double click to highlight the folder name and change the entry. Rename the file using the format MSNMMDDYYx, where N is instrument number, MM = month, DD = day, YY= year and x = a letter indicating the batch being run, e.g. MS3040315a. Click **OK** which will take you back to the sample log table.
3. In the Sample column verify the correctness of pre-filled entries. Starting at the first empty row, enter sample or QC information. For samples this would include aliquot number, laboratory number, specimen source, and dilution if any (i.e., 2-11-2432 fem). Enter the next sample in the batch in the next open field down the column.
4. In the Type column, select the corresponding sample type for each vial: Sample, Blank, Calibration.
5. In the Vial column, click in the cell with the number 1, hold down the left mouse button and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column and Increment. Verify that the vial numbers are correct, including washes and reinjections.
6. In the Method column, verify that the correct method is loaded in the first cell. Then click on the first cell containing the method name, hold down the left mouse and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column, No Increment. Verify that the method for each vial is correct.
7. In the Data File column, in the cell corresponding to the first vial, enter the data file name in the format MSNMMDDx001, where N = instrument number, MM = month, DD = day, and x = a letter indicating the batch being run, i.e. MS30403a001. Click on this cell, hold down the left mouse and drag to the last vial number of the sequence (the cells will be highlighted). Right click and select Fill Column and Increment. Verify that the data file information for each vial is correct.



8. In the Comment column, enter any additional information for the vials (e.g. lot numbers).
9. In the Multiplier column, enter any sample dilutions.
10. In the **Level** column, verify that the correct level numbers are entered for calibrators in this batch.
11. Verify **No Update** is selected for all vials under **Update Rf** and **Update Rt**.
12. Review the information typed for the sequence. Correct any information as needed. Verify that the Data Path is C:\MSDCHEM\1\Data\current sequence name. Verify that the Method Path is C:\MSDCHEM\1\METHODS. Then Click **ok**.

**Note:** Occasionally, it will be desirable to run several subsequences in one batch. Use the instructions below to accomplish this.

### **Setting-Up a Subsequence**

*On the Sample Log Table:*

1. Select "**Keyword**" for "Type"
2. Select "**DataPath**" for "Method/Keyword"
3. Under "Comment/KeywordString" type in the *new data path* for your subsequence ie: **C:\MSDCHEM\1\DATA\MSNMMDDYYy** where "N" is the instrument's number and "y" is the letter designated to the subsequence (***it must be different than that of the original sequence***).
4. The suffix of the data files must be different from that of the original; ie: MSNMMDDy001; ***the subsequence data files must start with 1 again.***
5. After typing in the entire sequence, save sequence accordingly.
6. Go to Sequence → Simulate Sequence → Run Sequence.
7. A dialog box will pop-up: DataPath **C:\MSDCHEM\1\DATA\MSNMMDDYYy** does not exist. Edit Sample Log Table? → Click **No** if the sequence was set up correctly.
8. A 2<sup>nd</sup> dialog box will pop-up: Create **C:\MSDCHEM\1\DATA\MSNMMDDYYy**? → Click **Yes**.
9. A 3<sup>rd</sup> dialog box will pop-up: Sequence Verification Done! View it? → Click **Yes** or **No**.

### **Setting-Up a Subsequence with a Different Method**

*On the Sample Log Table:*

1. After inserting the DataPath keywords and Commands, Insert a Row.
2. Select "**Keyword**" for "Type"

3. Select "**MethodPath**" for "Method/Keyword"
  4. Check that the method for each sample is changed to the new method.
  5. Follow Steps 5-9 in the "**Setting-Up a Subsequence**" Section (see previous section).
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13. On the Method and Run toolbar, under **Sequence**, select **Run Sequence**. In the dialog box under **Sequence Comment** enter the initials of the individual who has entered the sequence in the **Operator Field** (e.g., MPM). Under **Data File Directory** verify that the data file path is C:\MSDCHEM\1\DATA\MSNMMDDYYx.
  14. Under **Sequence**, select **Save Sequence As**. Under File Name, type in the name of the folder that the batch will be saved to under DATA, MSNMMDDYYx (e.g. MS3040315a) Select **Save**. The extension ".s" will automatically be added.
  15. Under **Sequence** select **Print Sequence**. Verify that **Brief Format** is selected and click on **OK**. The sequence will be printed. Apply the chain of custody labels to the printed sequence list.
  16. Use the printed sequence list to load vials into the appropriate autosampler positions. Check vial information against the sequence list and ensure that the vial is inserted in the correct numeric position in the autosampler. Date and initial the chain of custody label on the sequence printout, listing any comments, transfers or exceptions.
  17. Under Sequence select **Run Sequence**. Verify that the Sequence comments and Data Field information are correct (i.e. verify that the proper sequence is loaded. If not, load the proper sequence). Click on **Run Sequence**.
  18. After the batch is finished, unload the vials. Compare the vial information to the sequence list as they are removed, to verify that the correct vial was in the correct position. Date and initial the sequence list when this is completed. Annotate discrepancies if necessary

## **DATA TRANSFER AND PROCESSING - EXPORTING RAW DATA FOR PROCESSING**

All processing and review of the processed data should be performed on a processing computer.

### **SAVE METHOD TO ECM**

1. After the run finishes, the data files will be in the data subdirectory on the local chemstation and also automatically transferred to ECM. From the acquiring computer, make sure the proper method, the one used to acquire the data, is loaded. On the top toolbar under ECM click on Save Method to ECM.
2. Click on the GCMS, the correct instrument name folder, the appropriate month and batch to which the method will be saved.

## **RETRIEVE BATCH FROM ECM - RAW DATA FOR PROCESSING**

1. At the processing computer, click on Processing Data Analysis. Log on using your OCME network username and password.
2. On Enhanced Data Analysis screen, click on ECM at the top toolbar and select Retrieve entire sequence from ECM.
3. This will open up Openlab ECM screen. Select GCMS, then the appropriate instrument, the month, and, finally the batch to be retrieved. On the status line at the bottom of the screen that the batch is being retrieved. The batch will be downloaded to the following location: C:\msdchem\1\ECM\Retrieve\MSNMMDDYYx.

## **DATA REVIEW**

There are three levels of review; the first level of review is the transference and processing of the raw data, this may be performed by any trained analyst; the second level of review is performed by an experienced analyst who is trained and signed off in data review, he / she will review the processed data; the third level of review is considered the final level of review, this can only be performed by the Laboratory Managers. He/she will review the data for the entire case ensuring that screening, confirmatory and quantitative analysis on the case have been completed and reported accurately. As needed, he/she will also schedule additional analysis and contact the Medical Examiner on the case to discuss any findings and / or review case history.

## **LOAD METHOD AND BATCH – FIRST LEVEL REVIEW**

1. On the left side of the screen, open C:\msdchem\1\ecm\retrieve
2. Under retrieve, click on the batch that was retrieved. Verify that the appropriate method is present in the batch.
3. To load the method, right click on the method and select load. This will bring up “Be sure changes are saved. Load now?” Click Yes. If the method is not present, load the method by retrieving the method from ECM.
4. Click on any file in the batch to load it.

## **PERFORM BATCH CALIBRATION – PROCESSING – FIRST LEVEL REVIEW**

Under enhanced data analysis:

1. Process the calibrators. Select Tools from the toolbar, DoLIST, and Quant, No Report (QT 1). Press Add, and OK. Select the files for this action to be performed on, in this case, calibrators only. Verify that the selected files are located in the correct subdirectory. Change the path if necessary. Click the → Arrow and Process.

2. Review the integrations of the targeted compounds for each calibrator; checking that the ion peaks are present and integrated correctly (i.e. the baseline is the most scientifically accurate one that can be drawn). Select View from the toolbar, QEDIT. Answer appropriately when prompted to save changes made to quantitation results when moving from file to file. Return to Data Analysis by selecting View from the toolbar, return to Data Analysis.
3. Update the existing calibration table (all levels). Select Calibrate, Update, Quick Levels Update. When prompted to clear responses, select YES. When asked to requant files before update, select NO. Select single data file/level option. Select the appropriate data file to associate with calibration level 1 (5 mg/L). Click OK. Repeat for remaining calibration levels (10, 25, 50, 100 mg/L). Select level 3 when prompted to update retention times.
4. Load the file associated with level 3 (25 mg/L), by selecting File, Load Data File. Select Calibrate, Update One Level. Do NOT requant. Select Update One Level, select only Replace Qualifier Ion Relative Responses, and choose the corresponding existing level ID (#3). Click Do Update.
5. Review the Compound database. Double click on the internal standard listed on the left to reveal the compounds quantitated with it. Select the calibration tab to reveal compound responses, calibration curves, and  $r^2$ . To disable a point on the calibration curve for a compound, delete its response from the table. Click OK or Cancel when review is complete.
6. Save Method before proceeding. Select Method from the toolbar, Save Method, make sure that the path is correct. Save to OpenLab ECM at this time.
7. Requantitate the calibrators with the updated calibration curve. Select Tools from the toolbar, DoLIST, Requant, no report (QT 2), Add, and OK. Remove any existing commands. Select files to process. Click the → Arrow and Process. Review with QEDIT. Check the responses, retention times and ion ratios.
8. Regression correlation coefficient ( $r^2$ ) for each analyte must be equal to or greater than 0.99.
9. Process controls and cases. Select Tools from the toolbar, DoLIST, Quant, No Report (QT 1), Add, and OK. Select appropriate files. Click the → Arrow and Process. Review with QEDIT. Verify multipliers/dilution factors are applied.
10. When review is complete, return to Data Analysis. Select report format by choosing Quantitate from the toolbar, Report Options. Check SIM style report and uncheck Internal Standards. Press OK.
11. To print reports, select Tools from the toolbar, DoLIST, Profile Quant w/o Calculations (QT 0,1,'P'), Add, and OK. Select files to print, click the → Arrow and Process.
12. Print the calibration table for the current batch by clicking **Calibrate** on the command line. Select **List, Calibrate Report** and click OK. The Calibration report will print to the screen. Review the  $r^2$  values, then right click on the screen report to print it.
13. Save files to ECM. Select ECM from the toolbar, select "Save multiple data files to ECM". Select all files.
14. Save method to ECM. Select ECM from the toolbar, Save Method to ECM. Make sure data path is correct.

## BATCH CLEAN UP

15. Select my computer. Find the batch on the C drive at C:\msdchem\1\ecm\retrieve\batch. Right click on the batch to be deleted and select delete. Do not delete a batch that has not been successfully uploaded to ECM.

## ACCEPTANCE CRITERIA

1. Acceptance range for calibrators is  $\pm 20\%$  of the target concentration. A maximum two out of five calibrators may be dropped if outside of the acceptable range. However, the remaining acceptable calibrators must be re-processed and quantitative values for cases reported within the dynamic range of the acceptable calibration range.
2. Blood controls must be within  $\pm 20\%$  of the target value and must pass integration, retention time ( $\pm 2\%$  of the calibrator retention times), and peak shape criteria. The blank must not contain detectable amounts of target analytes or significant interfering peaks.
3. Ion ratios must be within  $\pm 20\%$  of the target ion ratio, as determined by the midpoint calibrator, for blood samples. For non-blood samples, the ion ratios may be accepted within  $\pm 30\%$ , if the analyte has met acceptance criteria in a blood matrix

**Note:** In some instances ratios will be off in exceptionally low or high concentrations. The operator must evaluate this and schedule proper dilutions or other methodologies, as needed. See REPORTING section.

4. The analyst shall choose from the standard levels a level at their discretion. All internal standard areas/peak heights for that sequence shall be compared to the internal standard(s) in that level. The acceptance criteria for the internal standard recovery/response shall be  $\pm 30\%$  of the selected internal standard. If the internal standard recovery/response is outside these limits the specimen shall be repeated unless the analyte in question is not related to the internal standard that is outside the acceptable limits. If the internal standard is still unacceptable in the repeat analysis in the same way (i.e. both times too high or both times too low), then the sample shall be repeated a third time using a "blank and spike" method. The "blank and spike" run shall be viewed as acceptable if the following conditions are met: All standards and QC materials are within acceptable ranges, the "blank and spike" samples internal standards are within  $\pm 20\%$  of their average responses, the calculated amount of the spiked sample is  $\pm 20\%$  of the theoretical amount. If any of these conditions are not met than consult with a toxicology manager for further direction.
5. Make copies of all controls, the  $r^2$  report and the sequence list, enough to attach a set to each case in the batch.
6. Enter the sequence with tune attached in the sequence logbook for the appropriate MS.

## SECOND LEVEL REVIEW

The Second Level Reviewer will review the processed data in its entirety according to the acceptance criteria section of this SOP.

## **REPORTING**

After the batch has undergone second level review and has been printed, either the first level reviewer or the second level reviewer may report the data in the appropriate case file

Reportusing the following guidelines:

1. Each case printout must have a copy of the sequence and all controls appended.
2. Concentrations greater than or equal to 10.0 mg/L are reported in mg/L. Results are reported to the tenth of a mg/L (ex: 10.275 mg/L is reported as 10.2 mg/L).
3. Concentrations less than 10.0 mg/L are reported as “not detected”.
4. Sample concentrations greater than the highest acceptable calibrator must be re-extracted with suitable dilution(s) to bring it within the calibration curve limits.
5. If the positive matrix controls are greater than  $\pm 30\%$  of target for non-blood matrices, the samples may be reported qualitatively, as detected or not detected. If quantitative results are needed, the sample must be repeated.
6. Submit the case file with all reports for Third Level Review.

## **THIRD LEVEL REVIEW (FINAL REVIEW)**

The third and final level review can only be performed by the Laboratory Managers. He/she will review the data for the entire case according to all established criteria. They will ensure that screening, confirmatory and quantitative analysis on the case have been completed and reported accurately. As needed, they will also schedule additional analysis and contact the Medical Examiner on the case to discuss any findings and / or review case history.

## **REFERENCES**

Clark's Identification and Isolation of Drugs in Pharmaceuticals, Body Fluids and Post-Mortem Materials, Ed. A.C. Moffat, The Pharmaceutical Press, London, Second Edition, 1986.

Agilent 6890 GC System Installation Guide.

Agilent 6890 GC System Users Guide.

Agilent 6890 GC System Standard Operating Procedures.

Agilent 5973 & 5973 Network Mass Selective Detector Installation Guide.

Agilent 5973 & 5973 Network Mass Selective Detector Users Guide.

Agilent 5973 & 5973 Network Mass Selective Detector Standard Operating Procedures.

United Chemical Technologies Procedure Manual

Uncontrolled Copy

## REVISION HISTORY

- |                |  |
|----------------|--|
| Ver 03.08.2013 | 1. Revision history implemented.   |
| Ver 04.20.2015 | 1. Modification of method to precondition spe cartridge by gravity.                  |
| Ver 08.31.2015 | 1. Added internal standard acceptance criteria.<br>2. Defined the level of review(s) |

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