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

# ETHYLENE GLYCOL and PROPYLENE GLYCOL by GAS CHROMATOGRAPHY/MASS SPECTROMETRY

# PRINCIPLE

Ethylene glycol (HOCH<sub>2</sub>CH<sub>2</sub>OH) is a relatively nonvolatile liquid used as a major component of antifreeze fluids. Propylene glycol is used extensively as a substitute for ethylene glycol in automotive antifreeze. Its pharmaceutical applications include use as a preservative, emollient and vehicle for both oral and intravenous medications.

Analysis of ethylene glycol and propylene glycol should be preformed in each case where ingestion of antifreeze is indicated. Propylene glycol analysis should be performed when case history indicates its pharmaceutical use.

# SAFETY

The handling of all reagents, samples and equipment is performed within the guidelines which are detailed in the safety manual.

# **REAGENTS AND MATERIALS**

- 1. Ethylene glycol (EG) (HOCH<sub>2</sub>CH<sub>2</sub>OH) (Fisher ACS or equivalent)
- 2. Propylene glycol (**PG**) (HOCH<sub>2</sub>CHOHCH<sub>3</sub>) (Fisher ACS or equivalent)
- 3. 1,3-Propanediol (trimethylene glycol) (HOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OH), internal standard. (Fisher ACS or equivalent)
- 4. Deionized water.
- 5. Certified negative blood.
- 6. Assorted glassware.
- 7. Vortex.
- 8. Acetonitrile. (Fisher ACS or equivalent)
- 9. Ethyl acetate. (Fisher ACS or equivalent)

# CALIBRATORS AND CONTROLS (ETHYLENE GLYCOL/PROPYLENE GLYCOL)

To determine the volume of Ethylene or Propylene glycol used in a calibrator or control, the following formula is used:

Density (d) = 
$$\frac{Mass(m)}{Volume(v)}$$
 or  $Volume = \frac{Mass(m)}{Density(d)}$ 

Calibrators and controls are prepared in matrix-matched negative matrix (deionized  $H_2O$  for urine and gastric contents) by using a 1:5 standard solution. The standard solution is prepared by adding 5 mL of either EG or PG to a 25 mL volumetric flask with a top. The flask is then filled to the line with deionized water; the top is placed in the flask, and the flask is inverted several times to mix the dilution.

The calibration curve for a glycol quant is prepared by spiking the following values:

# Calibrators

2000 mg/L - 18  $\mu$ L E.G. in 2 mL of blood (or H<sub>2</sub>O), 19.3  $\mu$ L P.G. in 2 mL of blood (or H<sub>2</sub>O) 1500 mg/L - 13.5  $\mu$ L E.G. in 2 mL of blood (or H<sub>2</sub>O), 14.45  $\mu$ L P.G. in 2 mL of blood (or H<sub>2</sub>O) 1000 mg/L - 9  $\mu$ L E.G. in 2 mL of blood (or H<sub>2</sub>O), 9.65  $\mu$ L P.G. in 2 mL of blood (or H<sub>2</sub>O) 500 mg/L - 4.5  $\mu$ L E.G. in 2 mL of blood (or H<sub>2</sub>O), 4.85 $\mu$ L P.G. in 2 mL of blood (or H<sub>2</sub>O) 100 mg/L - 0.9  $\mu$ L E.G. in 2 mL of blood (or H<sub>2</sub>O), 0.95 $\mu$ L P.G. in 2 mL of blood (or H<sub>2</sub>O)

(Specific gravity Ethylene Glycol = 1.112) (Specific gravity Propylene Glycol = 1.036)

# Controls

1200 mg/L - 11  $\mu$ L in 2 mL of blood (or H<sub>2</sub>O) EG, 11.6  $\mu$ L in 2 mL of blood (or H<sub>2</sub>O) PG 600 mg/L - 5.5  $\mu$ L in 2 mL of blood (or H<sub>2</sub>O) EG, 5.8  $\mu$ L in 2 mL of blood (or H<sub>2</sub>O) PG 200 mg/L - 1.8  $\mu$ L in 2 mL of blood (or H<sub>2</sub>O) EG, 1.9  $\mu$ L in 2 mL of blood (or H<sub>2</sub>O) PG

Note: Please vortex each fortified tube after spiking it with E.G. or P.G.

# Internal standard 500 mg/L

12  $\mu$ L of 1,3-propanediol 98% pure standard (specific gravity = 1.060) in 25 mL of acetonitrile.

# PROCEDURE

1. Place 100  $\mu$ L of calibrators, controls, and specimens in a properly labeled 10 mL conical centrifuge tubes.

**NOTE:** It has been reported that the internal standard, 1,3-propanediol, has been found as a component of some commercial antifreezes. Include one aliquot of each case without internal standard to check for the presence of this compound in the sample.

**NOTE:** If gastric contents are being tested, run undiluted and dilute 1:10 in  $H_2O$ . Blood should be analyzed undiluted and with a 1:2 dilution. Other specimens should be diluted as indicated.

- 2. Add 200 µL of acetonitrile containing the internal standard, 1,3-propanediol.
- 3. Vortex and centrifuge at  $\approx$  3000 rpm for 10 minutes to separate proteins.
- 4. Transfer all supernatant, which contains the ethylene glycol/propylene glycol and the internal standard, to autosampler vials.
- 5. Inject 3.0 µL of reaction mixture into the GC/MS. Injection volume can vary.
- 6. The specimens are first screened using a five-point calibration curve. Any positive specimens are repeated in a second batch.

# INSTRUMENTATION

Hewlett-Packard 6890 gas chromatograph with 5973 mass spectrometer equipped with a 7683A autosampler with controller and RTX-BAC1 - Blood Alcohol Column (30m x 0.32mm x 1.8um film thickness).

# TOP LEVEL PARAMETERS

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

Method Sections To Run:

(X) Save Copy of Method With Data

- () Pre Run Cmd/Macro =
- (X) Data Acquisition
- () Data Analysis
- () Post-Run Cmd/Macro =

Method Comments:

Glycols by GCMS

#### **INSTRUMENT CONTROL PARAMETERS**

Sample Inlet	GC	
Injection Source	GC ALS	
Mass Spectrometer	Enabled	

#### HP6890 GC METHOD

#### OVEN

Initial temp	70°C (On)	Maximum temp	295 °C
Initial time	4.00 min	Equilibration time	3.00 min

Ramps			
#	Rate	Final temp	Final time
1	30	190	5.17
2	0.0(Off)		
Post temp	190°C		
Post time	2.00 min		
Run time	13.17		
	min		

INLET

Front Inlet (Unknown)		
Mode	Split	
Initial temp	250 °C (On)	
Pressure	9.03 psi (On)	
Split ratio	10:1	
Split Flow	10.0 mL/min	
Total flow	14.2 mL/min	
Gas saver	on	

Saver flow	20.0 ml/min
Saver time	3.5 min
Gas type	Helium

# COLUMN

Column 1		Column 2	
Capillary Column		(not installed)	
Model Number:	Restek RTX-BAC1		
Max temperature	240 °C		<b>A</b>
Nominal length	30.0 m		
Nominal diameter	320.00 μm		
Nominal film thickness	1.80 μm		
Mode	Constant flow		
Flow	1.0 ml/min		
Nominal initial pressure	9.03 psi		
Average velocity	17 cm/sec		
Inlet	Front Inlet		
Outlet	MSD		
Outlet pressure	vacuum		

# DETECTOR

Outlet pressure	vacuum			
CTOR			0	
FRONT DETECTOR (NO	) DET)	BACK DE	TECTOR (NO	DET)
COLUMN COMP 1		COLUMN	COMP 2	
(No Detectors Installed)		(No Detec	tors Installed)	
L		$\mathbf{O}$		

# SIGNAL

Signal 2
Data rate: 20 Hz
Type: test plot
Save data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
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	2.0 min
Oven Temperature	190 °C
Column 1 flow	2.0 ml/min

#### 7673 INJECTOR

Front Injector:	
Sample Washes	1
Sample Pumps	4
Injection Volume	3.0 microliters (can vary)
Syringe Size	10.0 microliters
PreInj Solvent A Washes	1
PreInj Solvent B Washes	1
PostInj Solvent A Washes	5
PostInj Solvent B Washes	5
Viscosity Delay	0 seconds
Plunger Speed	Fast
PreInjection Dwell	0.00 minutes
PostInjection Dwell	0.00 minutes

*Back Injector:* No parameters specified

#### **MS ACQUISITION PARAMETERS**

General Information		
Tune File	lowmass.u	
Acquisition Mode	Scan	
MS Information		
Solvent Delay	2.0 min	
EM Absolute	relative	
EM Offset	0	
Resulting EM Voltage	(varies)	
[Scan Parameters]		
Low Mass	25.0	
High Mass	175.0	
Threshold	150	
Sample #	2	
Plot 2 low mass	25.0	
Plot 2 high mass	175.0	

[MSZones]

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

# **INSTRUMENT SETUP**

Perform a mass spectrometric (MS) tune to check if the instrument is operable, using the low mass autotune procedure. See the "Mass Spectrometric Tuning (Autotune)" SOP for specifications.

An acceptable autotune must be obtained prior to batch analysis.

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 and relog in. Using the Chemstation software, at the top Method and Run toolbar under **ECM**, select **Logon 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. MS3041111a. Click OK which will take you back to the sample log table.
- 3. In the Sample column verify the correctness of pre-loaded entries. Starting at the first empty field, enter sample or QC information. For samples this would include aliquot number, laboratory number, specimen source, dilution if any (i.e., 2-11-2432 fem). If the sample has been diluted, enter the appropriate dilution factor in the Multiplier column. For QC samples use an appropriate designator. 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 or QC.
- 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.
- 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. MS20411a001. 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.
- 9. In the Multiplier column, enter any sample dilutions.
- 10. Verify No Update is selected for all vials under Update Rf and Update Rt.

- 11. 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.
- 12. Go to Sequence. Select Simulate Sequence. Enter in the comment field whether a glycol quant or screen created and enter your initials in the operator field. Save the sequence accordingly.

**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"
- Under "Comment/KeywordString" type in the new data path for your subsequence i.e.: C:\MSDCHEM\1\DATA\MSNMMDDYYx where "N" is the instrument's number and "x" 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; i.e.: MSNMMDDy001; *the subsequence data files must start with 1 again*.
- 5. After typing in the entire sequence, save sequence accordingly.
- 6. Go to Sequence  $\rightarrow$  Simulate Sequence  $\rightarrow$  Run Sequence.
- 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.
- 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?  $\rightarrow$  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).

- 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\batch name.
- 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. MS2041211a) 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 preprinted labels for documenting verification of process steps to the printed sequence list.
- 16. Use the printed sequence list to load vials into the appropriate autosampler positions as indicated by the order on the printed sequence list. Check vial information against the sequence list and ensure that the vial is inserted in the correct numeric position in the autosampler as indicated on the sequence list. Document correct positioning by dating and initialing the appropriate line on the sequence list.
- 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 must 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.
- 3. You may first need to log off and then log on to Chemstation at the acquiring computer.

# **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\"batch name"

# 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 Manager. 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.

# PROCESSING USING ENHANCED DATA ANALYSIS - FIRST LEVEL REVIEW

On any processing station:

- 1. Click on the Processing Data Analysis icon on the desktop and 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\"batch name"
- 4. Select the method under the batch by right clicking on it and Load the method.
- 5. Select Calibrate from the tool bar, then Update, Global Update, Set Curve Fit Type, Linear Regression Force (0,0), select OK.
- 6. Load the calibrator at the highest level (i.e. 2000mg/l) by selecting File $\rightarrow$  Load Data File.
- 7. Select View from the top menu, select Easy ID (this function allows for Retention times to be assigned for the entire method and batch). Easy ID should only be used for the first calibrator level.
- 8. To select a compound from the list double click; use the right click to integrate a peak for the compound, use the left click to control zooming on the chromatogram.
- 9. Once all of the retention times have been assigned, click on Exit and click on Yes to update retention times to compound list.
- 10. In the space provided type in QT 1, then click on Execute; this processes the data file.
  - 1. Select View from the top menu, select Q-Edit Quant Result (this function is to review the response, retention times and ion ratios for each compound). Click Exit when all compounds have been reviewed, and choose Yes to save changes made to quantitation results.

- 2. Assign the calibrator level:
- 3. Select Calibrate, then Update, select Update 1 Level.
- 4. Select Update Level, then Responses, select Replace.
- 5. Choose calibrator level to be updated (i.e. 5 for 2000mg/l; 4 for 1500mg/l...).
- 6. Click on Do Update then select OK.
- 11. Save the method.
- 12. Load the next data file, 1500mg/l. Type QT 1 then click on Execute.
- 13. Select View; select Q-Edit Quant Result. *Easy ID is not needed for this step, as retention times have already been set for this calibrator.* Double check each of the compound's retention time, response and ion ratios. Click Exit once when finished.
- 14. Assign the calibrator level.
- 15. Repeat steps 10-14 for the remaining levels for the calibrator.
  - 1. Requant the calibrator pool:
  - 2. Go to Tools, DOLIST.
  - 3. Remove any options that are on the right hand side of the box.
  - 4. On the left hand menu select "Requant, no report", which is QT 2, with arrows move this command to the empty space on the right then click OK.
  - 5. Select the calibrator pool just processed and move it to the empty space on the right then click Process. Each file will be requanted.
- 16. Review all the files through Q-Edit Quant Result to verify quant results.
  - 1. Print the calibrators:
  - 2. Go to Tools, DOLIST.
  - 3. Remove any options that are on the right hand side of the box.
  - 4. On the left hand menu select Full Quant w/o Calculations which is QT 0,1,'F' with arrows move this command to the empty space on the right, then click OK.
  - 5. Only select all Cal levels just reviewed and quanted and move them to the empty space on the right, then click Process. Each file will be processed and generate a report.
- 17. Save the method.
- 18. Process the blank, corresponding QC's and cases for the calibrator curve:
  - 1. Go to Tools, DOLIST.
  - 2. Remove any options that are on the right hand side of the box.
  - 3. On the left hand menu select "Quant No Report" which is QT 1 with arrows move this command to the empty space on the right, then click OK.
  - 4. Select the blank, QC's and cases to be processed and move them to the empty space on the right then click Process. Each file will be processed.
- 19. Review each blank, QC and case by selecting View, select Q-Edit Quant Result. For each blank and case, deselect any compounds that are not needed by double clicking on it and clicking on QDel. For each blank, QC and case, check the responses and ion ratios of the needed compounds.

- 20. Print the blank, QC's and cases:
  - 1. Go to Tools, DOLIST.
  - 2. Remove any options that are on the right hand side of the box.
  - 3. On the left hand menu select Full Quant w/o Calculations, which is QT 0,1, 'F', with arrows move this command to the empty space on the right then click OK.
  - 4. Only select the blank, QC's and cases just reviewed and quanted and move them to the empty space on the right then click Process. Each file will print.
- 21. Print an r<sup>2</sup> report by:
  - 1. Go to Calibrate, Print Calibration Curve. Select ethylene glycol. Then select propylene glycol.
- 22. Once everything is printed, save all the data files from the batch back to ECM:
  - 2. Select ECM from the top tool bar, select Save Multiple Date Files to ECM.
  - 3. Select all data files on the left and move them to the right empty space, then click Process.
  - 4. Allow each data file to be successfully copied to ECM before exiting Data Analysis.

# BATCH CLEAN UP

1. 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**

All chromatograms must be reviewed according to the following guidelines:

1. Quantitation ion and qualifier ions listed in the method must be present.

Ethylene glycol	31, 43, 62
Propylene glycol	45, 61, 29
1,3-Propanediol	57, 28, 43

- 2. A five point calibration curve is established using 100, 500, 1000, 1500, 2000 mg/L calibrators. One calibrator may be dropped if appropriate acceptance criteria are not met.
- 3. Linear regression correlation coefficient must be  $\geq 0.98$ .
- 4. All positive blood matrix controls must be within  $\pm$  20% of the target concentration.

All positive water matrix controls must be within  $\pm$  30% of the target concentration.

- 5. All negative matrix controls must be negative.
- 6. Retention times must be within  $\pm 2\%$  of the calibrator retention times.
- Ion ratios must be within ± 20% of the average of the calibrators for blood or 20% of the nearest calibrator. For tissues, ± 30 % is acceptable. If these ratios are exceeded, this drug is not to be considered a match.

**NOTE:** Sometimes ratios will be off in exceptionally low or high concentrations. The operator must evaluate this and schedule proper dilutions or other methodologies, as needed.

- 8. Peak shape must be symmetrical and Gaussian in appearance.
- 9. Sample concentrations in excess of the highest calibrator must be re-extracted with a larger dilution with appropriate matrix controls.
- 10. If the aliquot of the sample without internal standard has a peak at 1, 3-propanediol, schedule the case to be repeated. Consult with supervisor in these cases.
- 11. 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 ± 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 ± 20% of their average responses, the calculated amount of the spiked sample is ± 20% of the theoretical amount. If any of these conditions are not met than consult with a toxicology manager for further direction.

# 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.

# Report using the following guidelines:

- 1. Evaluate and review data in accordance with guidelines in the Acceptance Criteria section.
- 2. The positive matrix controls must be signed and dated by reviewer. Any notations as to passing or failing values must be noted on the positive matrix controls.
- 3. The positive matrix controls must be copied and attached to all casework from that batch.
- 4. Concentrations of ethylene glycol or propylene glycol below 50 mg/L are reported as "not detected".
- 5. Concentrations of ethylene or propylene glycol below 100 mg/L and greater than 50 mg/L are reported as "less than 100 mg/L".
- 6. Concentrations greater than 100 mg/L are reported to one decimal point in mg/L.

- 7. Since this method uses full scan GCMS, other peaks may be identified by library match. If another glycol is identified, discuss with supervisor, and request a standard of that compound to be acquired for verification of identity and quantitation.
- 8. 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 Manager. 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

This method was developed in-house.

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.

# **REVISION HISTORY**

Ver 03.08.2013 Ver 05.07.2013 Ver 07.02.2014	1. 1. 2. 1. 2. 3.	Revision history implemented. Added 200 mg/L QC Moved Directors signature from bottom of each page to the final page of the SOP. Added how to prepare EG and PG standard solutions Added a step to prepare a sequence. Changed step 20 in processing using enhanced data analysis. Removed signature page to original format Minor grammatical & spelling corrections. Initial screening changed from a single point calibration to
Ver 04.20.2015	4. 1. 2.	
Ver 08.31.2015	1. 2.	Added internal standard acceptance criteria. Defined the level of review(s)