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

CARISOPRODOL , MEPROBAMATE and TOPIRAMATE by SOLID PHASE EXTRACTION and GAS CHROMATOGRAPHY/MASS SPECTROMETRY (Selected Ion Monitoring)

PRINCIPLE

Carisoprodol is a carbamate derivative first synthesized in 1959. It is primarily used as a muscle relaxant. Meprobamate is also a carbamate derivative used as a muscle relaxant and the primary metabolite of carisoprodol. Topiramate is a sulfamate-substituted monosaccharide used as an anticonvulsant. Carisoprodol, meprobamate and topiramate are quantitated by a selected ion monitoring (SIM) method using methapyrilene as the internal standard.

Carisoprodol, meprobamate and topiramate are extracted from biological specimens by solid phase extraction. 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 drugs from the column. The eluate is evaporated, reconstituted and injected in the GCMS. Quantitative analysis is performed by SIM GCMS using a six point calibration curve.

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	0.5 mL of the undiluted specimen
Urine	0.5 mL for qualitative identification
Brain	0.5 mL of a 1:3 homogenate
Gastric Contents	0.5 mL of a 1:10 dilution
Liver	0.5 mL of a 1:5 homogenate
Vitreous Humor	0.5 mL of the undiluted specimen
Bile	0.5 mL of the undiluted specimen

Dilution of Specimens

Specimens are diluted as follows:

Brain 1:3	5.0 g of brain homogenized with 10 mL of distilled water.
Liver 1:5	5.0 g of liver homogenized with 20 mL of distilled water.
Gastric 1:10	2.0 mL of liquid *q.s.* to 20 mL of distilled water, or 2.0 g of a solid specimen homogenized with 18 mL of distilled water.

Note: *Use a homogenate which was prepared within two weeks. Do not use homogenates older than two weeks unless low sample size requires it. Discuss with supervisor and note in case record. The entire submitted amount of gastric contents needs to be homogenized prior to sampling.*

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. **Negative blood, serum, brain, liver**

   Calf or sheep blood obtained from outside source or equivalent. Sodium fluoride is added as a preservative, and stored frozen (-10EC or lower). Human plasma/serum obtained from outside source or equivalent, and stored frozen (-10EC or lower). Calf brain and liver obtained from outside source or equivalent. Homogenized and stored frozen (-10EC or lower). All matrices are validated as negative by in-house analysis
4. **100 mM Sodium Acetate buffer (pH 4.5)**

   Dissolve 11.72 g sodium acetate trihydrate in 1800 mL DI H_2O; add 6.5 mL glacial acetic acid. Dilute to 2000 mL using DI H_2O. Mix. Adjust pH to 4.5 ± 0.1 with sodium acetate or acetic acid.
   Storage: Room temperature in glass.
   Stability: 6 months. Inspect daily with use for contamination.
5. **pH 9.0 buffer**

   Dissolve 40 g $KHCO_3$ in 1600 mL DI H_2O; add 20 g K_2CO_3. Dilute to 2000 mL using DI H_2O. Mix. Adjust pH to 9.0 ± 0.1 with $KHCO_3$ or K_2CO_3.
   Storage room temperature in glass.
   Stability: 6 month. Inspect each day of use contamination.
6. **Eluting solvent**: Ethyl acetate:NH_4OH (98:2). Prepare fresh each day of use.
7. **Polycrom Clin II Solid Phase Extraction Column**, CEREX, SPEware.
8. **System 48 Processor** connected to nitrogen source.
9. **Waste Rack, SPE Rack, Collection Tube Rack.**

10. **Evaporation apparatus** (e.g. Turbovap or CEREX concentrator) connected to a nitrogen source
11. **Sonicator**
12. **Vortex**
13. **Centrifuge**
14. **GCMS column**, Restek RTX-50 30M x 0.25 mm ID x 0.25 µm DF, Model # 10523
15. **Meprobamate, Carisoprodol and Topiramate calibrator solutions (100mg/L).**

    Carisoprodol working calibrator solution. (100 mg/L)
    Weigh 0.0100 grams of carisoprodol into a 100 mL volumetric flask and qs to 100 mL with methanol. Transfer to properly labeled containers.

    Meprobamate working calibrator solution. (100 mg/L)
    Pipet 1 mL of 1 mg/mL meprobamate reference standard into a 10 mL volumetric flask. QS to 10 mL with methanol. Transfer to properly labeled containers.

    Topiramate working calibrator solution (100 mg/L)
    Pipet 1 mL of 1 mg/mL topiramate reference standard into a 10 mL volumetric flask. QS to 10 mL with methanol. Transfer to properly labeled containers.

    Note: *Include identity, concentration, solvent, lot number, date prepared, and initials of analyst.*

16. **Meprobamate , carisoprodol and topiramate control solutions (100 mg/L).**

    Preparation is the same as meprobamate, carisoprodol and topiramate calibrator solutions as listed in step 15, using a different lot of primary standard (if available). If a different primary standard is not available, use a separate aliquot of the primary standard used to make the calibrator.

17. **Methapyrilene (MPY) Internal Standard, 50 mg/L.**

    See SOP section “Calibrators and Controls for Basic Drugs by GC and GCMS” for preparation.

PROCEDURE

1. Aliquot 0.5 mL of validated negative specimen into each tube labeled as calibrator or in-house control or 0.5 mL of sample into a 16 x 125 mm disposable culture tube labeled as to the contents.
2. Add appropriate amounts of standard solutions to negative matrix specimen tubes as follows:

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

   I. 0.5 mg/L - add 2.5 µL of 100 mg/L meprobamate, carisoprodol and topiramate calibrator solution

II. 1.0 mg/L - add 5.0 µL of 100 mg/L meprobamate, carisoprodol and topiramate calibrator solution

III. 1.5 mg/L - add 7.5 µL of 100 mg/L meprobamate, carisoprodol and topiramate calibrator solution

IV. 2.0 mg/L - add 10.0 µL of 100 mg/L meprobamate, carisoprodol and topiramate calibrator solution

V. 3.0 mg/L - add 15.0 µL of 100 mg/L meprobamate, carisoprodol and topiramate calibrator solution

VI. 5.0 mg/L - add 25.0 µL of 100 mg/L meprobamate, carisoprodol and topiramate calibrator solution

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

Additionally, two controls for verification of the calibrators are included in the run. Prepare a positive control of 0.5 mg/L by adding 2.5 µL of 100 mg/L topiramate and meprobamate and carisoprodol control solutions to the negative blank material. In addition, prepare a control of 2.5 mg/L by adding 12.5 µL of 100 mg/L topiramate and meprobamate and carisoprodol control solutions to the negative blank material. Prepare both controls in each matrix analyzed in the batch.

3. Add 15 µL of 50 mg/L MPY working internal standard solution to all tubes. The concentration of the internal standard in each sample is 1.5 mg/L.
4. Add 2.5 mL of DI H_2O and 2mL of pH 4.5 Sodium Acetate Buffer. Vortex for 15 seconds.
5. Centrifuge at ≈3000 rpm for 10 minutes.
6. Decant the supernatant into the Polychrom Clin II column and apply nitrogen at a pressure of 2-4 psi.
7. Wash column (All wash steps are pressurized at 2-4 psi)

   Pour 1mL pH 9.0 Buffer onto column

   Pour 1mL DI H_2O onto column.
8. Dry columns for 20 minutes at 25 Psig.
9. Prepare Elution Solvent

   Ethyl Acetate/NH_4OH (98/2). Mix well. Prepare fresh each day of use.
10. Elute Drugs

    Place labeled 10 mL conical centrifuge tubes under each column to collect eluate. Elute with 2.0 mL. Evaporate samples to dryness at with nitrogen.
11. Dry under nitrogen at 40 °C to absolute dryness.
12. Reconstitute the dried extracts with 100 µL of Ethyl Acetate.
13. Transfer extract to an insert placed in a labeled autosampler vial. 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. When using aluminum seals, ensure that the seal is tight by attempting to rotate the seal. Reseal, using a new aluminum cap, if necessary. Do not wait until all transfers have been made to seal the vials. Samples are ready for MS injection.

14. Create batch sequence as specified in Instrument Setup.
15. Enter the date completed in the Dataease database, so that the samples are not duplicated by another analyst.

INSTRUMENTATION

Agilent 5973 Mass Spectrometer with 6890 GC, Autosampler Agilent 7683, and Agilent Chemstation with current revision of software. The method name for this assay is MSNMCTSIM.M, where N is the instrument number. Each MSD used for Topiramate and Meprobamate and Carisoprodol analysis will have a method with this name in the method directory.

The following ions are monitored for each drug:

Meprobamate	83, 144, 114
Carisoprodol	158, 245, 184
Topiramate	324,189,110
MPY IS	97, 191

TOPLEVEL PARAMETERS

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

Method Sections to Run:

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

METHOD COMMENTS:

This is the caris./meprobamate/topiramate quant 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	140 °C (On)	Maximum temp	320 °C
Initial time	1.00 min	Equilibration time	0.50 min

Ramps			
#	**Rate**	**Final temp**	**Final time**
1	15.00	295	5.00
2	0.0(Off)		
Post temp	295 °C		
Post time	5.00 min		
Run time	16.33 min		

Front Inlet (Unknown)		*Back Inlet ()*
Mode	Pulsed Splitless	Not used
Initial temp	260 °C (On)	
Pressure	12.77 psi (On)	
Pulse Pressure	30.0 psi	
Pulse Time	0.80 min	
Purge flow	7.5 mL/min	
Purge time	0.00 min	
Total flow	10.3 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	
0.25mm * 15M * 0.25 µm		
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	12.77 psi	
Average velocity	38 cm/sec	
Inlet	Front Inlet	
Outlet	MSD	
Outlet pressure	Vacuum	

FRONT DETECTOR (NO DET)	*BACK DETECTOR (NO DET)*
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

COLUMN COMP 1	COLUMN COMP 2
(No Detectors Installed)	(No Detectors Installed)

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	5.00 min
Oven Temperature	295 °C
Column 1 Flow	1.2 mL/min

Time Table		
Time	**Specifier**	**Parameter & Setpoint**
7673 Injector		
Front Injector:		
Sample Washes	1	
Sample Pumps	2	
Injection Volume	5.0 microliters	
Syringe Size	10.0 microliters	
PreInj Solvent A Washes	1	
PreInj Solvent B Washes	1	
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	
Back Injector:		
No parameters specified		

MS ACQUISITION PARAMETERS

General Information	
Tune File	Atune.u

Acquistion Mode	SIM
MS Information	
Solvent Delay	5.0 min
EM Absolute	False
EM Offset	200
Resulting EM Voltage	(varies)

[Sim Parameters]

Group 1			
Group ID	1		
Resolution	High		
Plot 1 Ion	83.0		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(83.0, 300)	(97.0, 50)	(110.0, 50)
	(114.0, 300)	(144.0, 300)	(158.0, 10)
	(184.0, 10)	(189.0, 50)	(191.0 50)
	(245.0, 10)	324.0, 50)	

[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 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. The 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 MSNMMDDYYN, 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 MSNMMDDN001, 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\MSNMMDDYY*y*** 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: MSNMMDD**y**00**1**; ***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\MSNMMDDYY*y*** does not exist. Edit Sample Log Table? → Click **No** if the sequence was set up correctly.
8. A 2nd dialog box will pop-up: Create **C:\MSDCHEM\1\DATA\MSNMMDDYY*y***? → Click **Yes**.
9. A 3rd 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).

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 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 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 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 folder.
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 (0.5 mg/L). Click OK. Repeat for remaining calibration levels (1.0, 1.5, 2.0, 3.0, 5.0 mg/L). Select level 3 when prompted to update retention times.
4. Load the file associated with level 3 (1.5 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 0.98.
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 correctly.
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

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

1. Acceptance range for calibrators is ± 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 ± 20 % of the target value and must pass integration, retention time (± 2 % of the calibrator retention times), and peak shape criteria. The blank must not contain detectable amounts of target analytes or significant interfering peaks. For tissues, the controls are acceptable up to ± 30 % of the target value.
3. Ion ratios must be within ± 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 ± 30%, if the analyte has met acceptance criteria in a blood matrix.

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 ± 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.

5. Make sufficient copies of the controls, calibration report, and the sequence list, enough to attach a set for 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.

Report using 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 0.5 mg/L are reported in mg/L. Results are truncated and reported to one decimal point (e.g., 0.575 mg/L is reported as 0.5 mg/L).
3. Concentrations below 0.5 mg/L but meeting all other criteria are reported as "less than 0.5 mg/L". If the drug is not detected, or the criteria are not met, the drug is reported as "not detected".
4. Sample concentrations greater than the highest acceptable calibrator must be re-extracted with suitable dilution(s) to bring the concentration below the ULOL.
5. If the positive matrix controls are greater than ± 30 % of target for non-blood matrices, the samples may be reported qualitatively, as "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

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REVISION HISTORY

Ver 03.08.2013	1. Revision History implemented.
Ver 04.20.2015	1. Modification of buffer preparation (volumes) 2. Changed drying time to 20 minutes.
Ver 08.31.2015	1. Added internal standard criteria. 2. Defined the level of review(s)