PRINCIPLE

Nicotine is a potent parasympathomimetic alkaloid found in the nightshade family of plants. It is a major constituent of tobacco. In humans, nicotine is primarily metabolized to cotinine, which is further metabolized to trans-3′-hydroxycotinine. The major pathway of nicotine metabolism in humans is the C-oxidation to form cotinine, a two-step process. Cotinine may also be metabolized to 5′-hydroxycotinine, norcotinine, and cotinine N-oxide.

Specimens in which nicotine and cotinine are initially detected by the gas chromatography/Mass spectrometry (GC/MS) screening procedure are verified by a selective ion monitoring (SIM) GC/MS method using methapyriline as the internal standard.

Nicotine and cotinine 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 on the GCMS. Qualitative analysis is performed by SIM GCMS using a single point calibration curve.

SAFETY

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

REAGENTS AND MATERIALS

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

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1.0 mL of the undiluted specimen</td>
</tr>
<tr>
<td>Urine</td>
<td>1.0 mL for qualitative identification</td>
</tr>
<tr>
<td>Brain</td>
<td>1.0 mL of a 1:3 homogenate</td>
</tr>
<tr>
<td>Gastric Contents</td>
<td>1.0 mL of a 1:10 dilution</td>
</tr>
<tr>
<td>Liver</td>
<td>1.0 mL of a 1:5 homogenate</td>
</tr>
<tr>
<td>Vitreous Humor</td>
<td>1.0 mL of the undiluted specimen</td>
</tr>
<tr>
<td>Bile</td>
<td>1.0 mL for qualitative identification</td>
</tr>
</tbody>
</table>
Dilution of Specimens

Specimens are diluted as follows:

<table>
<thead>
<tr>
<th></th>
<th>Dilution</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1:3</td>
<td>5.0 g of brain homogenized with 10 mL of distilled water.</td>
</tr>
<tr>
<td>Liver</td>
<td>1:5</td>
<td>5.0 g of liver homogenized with 20 mL of distilled water.</td>
</tr>
<tr>
<td>Gastric</td>
<td>1:10</td>
<td>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.</td>
</tr>
</tbody>
</table>

**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 ACS grade or better.

1. Deionized water (distilled can be substituted)
2. Methanol (Fisher Scientific - ACS Certified)
3. Certified negative blood. Previously found to have no interfering peaks. Freeze validated negative blood. Discard after 3 months.
4. Certified negative liver. Previously found to have no interfering peaks. Freeze validated negative liver. Discard after 3 months.
5. Certified Negative Brain. Previously found to have no interfering peaks. Freeze validated negative brain. Discard after 3 months.
6. 100 mM phosphate buffer (pH 6.0)
   Dissolve 3.4 g Na₂HPO₄ and 24.28 g NaH₂PO₄·H₂O in 1600 mL DI H₂O. Dilute to 2000 mL using DI H₂O. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).
   Storage: room temperature in glass.
   Stability: Six (6) months. Inspect each day before use for contamination.
7. 200 mM HCL.
   Add 4.2 mL of concentrated HCL to about 100 mL of deionized water in a 250 mL flask.
   QS. to 250 mL with deionized water.
8. Hexane (Fisher Scientific- ACS Certified)
9. Polycrom Clin II Solid Phase Extraction Column, CEREX.
10. System 48 Processor connected to nitrogen source.
12. Evaporation apparatus (e.g., Turbovap or CEREX concentrator) connected to a nitrogen source.

13. Methapyriline (internal standard), Cerilliant or equivalent, (50 mg/L). Weigh 0.114 g methapyrilen HCl per 100 mL final volume (1000 mg/L), and prepare as indicated above to produce a stock solution of 1000 mg/L. Store stock solution frozen at -10\(^\circ\) C or lower. Stable for at least one year. Dilute 1:20 with methanol to produce working internal standard solution. Stored at 2 -8\(^\circ\) C. Stable for at least one year.

14. Weigh 0.0312 g nicotine, (hydrogen tartrate salt, Sigma Aldrich or equivalent) and add to 100 mL volumetric flask. Add 1mL of 1 mg/mL cotinine (Cerilliant or equivalent) ampoule to the 100 mL volumetric flask. QS.to 100 mL using methanol (99% pure or equivalent). Final concentration is 10 mg/L in methanol.

15. Nicotine and cotinine working calibrator and control solutions are prepared in same manner and transferred to a properly labeled container including lot number, concentration, date prepared, initials of analyst, etc. Store at 2-4\(^\circ\) C.

**EXTRACTION PROCEDURE**

1. Aliquot 1 mL of validated negative matrix (in each tube labeled as calibrator or in-house control) or sample into a 16 x 125 mm disposable culture tube labeled as to the contents.

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

2. Add appropriate amounts of standard solutions to negative matrix specimen tubes as follows:

   A single point calibrator at 0.5 mg/L and appropriate matrix matched blank(s) (IS only) should be run with each batch of samples.

   Calibrators are prepared in the following concentrations:

   - 0.5 mg/L – add 50 \(\mu\)L of 10.0 mg/L nicotine/cotinine calibrator solution

   Matrix matched controls are prepared as follows:

   - 0.01 mg/L – add 1 \(\mu\)L of 10 mg/L nicotine/cotinine control solution
   - 0.05 mg/L – add 5 \(\mu\)L of 10 mg/L nicotine/cotinine control solution
   - 0.2 mg/L – add 20 \(\mu\)L of 10 mg/L nicotine/cotinine control solution

3. Add 25 \(\mu\)L of methapyriline internal standard solution (50 mg/L) to each tube.

4. Add 2 mL 100 mM phosphate buffer (pH 6.0)

5. Vortex for 30 secs and sonicate for 10 minutes.

6. Centrifuge at \(\approx\) 3000 rpm for 10 minutes.
7. Pour the supernatant into the appropriately labeled columns and apply nitrogen to achieve a pressure of 2-4 psi.

8. Wash column (All wash steps are pressurized at 2-4 psi).
   a. Pour 2ml DI H₂O onto column
   b. Pour 2ml 200 mM HCL onto column
   c. Dry columns for 10 minutes at 25 Psig
   d. Pour 2ml of hexane onto column
   e. Dry columns for 5 minutes at 25 Psig
   f. Pour 2ml methanol onto column
   g. Dry columns for 5 minutes at 25 Psig
   h. Prepare Elution Solvent
      b. Pour 20 mL of isopropaol to glass flak with a stopper. Add 2mL of refrigerated Ammonium Hydroxide. Mix well by swirling and slowly add 78 ml. of methylene Chloride, mixing the solution by gentle inversion of flask.
      Note: Prepare fresh elution solvent each day of use.

9. Elute Drugs
   Place labeled 10 mL conical centrifuge tubes under each column to collect eluate. Elute with 2.0 mL; collect eluate at 1 to 2 mL/minute.

10. Dry using the concentrator, at room temperature, to absolute dryness.

11. Reconstitute the dried extracts with 100 μL of Ethyl Acetate.

12. Label autosampler vials indicating aliquot and toxicology number (ex: 1-YY-xxxx), specimen type, dilution, analyst, and date.

13. Transfer extract to an insert placed in an 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.


15. Enter the date completed in the Dataease database, so that the samples are not duplicated by another analyst.

**INSTRUMENTATION**

Agilent 6890 gas chromatograph with HP5973 mass spectrometer equipped with a 7683A auto-sampler with controller and a Restek RTX-BAC 1 (propriety Crossbond phase). 30M x 0.32 mm ID x 1.8 μm DF, max temperature 260 °C. Model # 18003
TOP LEVEL PARAMETERS

Method Information For: C:\HPCHEM\METHODS\MSXNICOTINT.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:

NICOTINE AND COTININE METHOD – MSXNICOTINT.M

INSTRUMENT CONTROL PARAMETERS

<table>
<thead>
<tr>
<th>Sample Inlet</th>
<th>GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection Source</td>
<td>GC ALS</td>
</tr>
<tr>
<td>Mass Spectrometer</td>
<td>Enabled</td>
</tr>
</tbody>
</table>

HP6890 GC METHOD

OVEN

<table>
<thead>
<tr>
<th>Initial temp</th>
<th>120 °C (On)</th>
<th>Maximum temp</th>
<th>260 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial time</td>
<td>1.00 min</td>
<td>Equilibration time</td>
<td>0.50 min</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Ramps</th>
<th>Rate</th>
<th>Final temp</th>
<th>Final time</th>
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<tbody>
<tr>
<td>1</td>
<td>20.00</td>
<td>260</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>0.0(Off)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Post temp 260 °C
Post time 5.00 min
Run time 10.75 min

Front Inlet (Unknown)  Back Inlet ()
<table>
<thead>
<tr>
<th>Mode</th>
<th>Pulsed Splitless</th>
<th>Not used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial temp</td>
<td>260 °C (On)</td>
<td></td>
</tr>
<tr>
<td>Pressure</td>
<td>1.68 psi (On)</td>
<td></td>
</tr>
<tr>
<td>Pulse Pressure</td>
<td>30.0 psi</td>
<td></td>
</tr>
<tr>
<td>Pulse Time</td>
<td>0.50 min</td>
<td></td>
</tr>
<tr>
<td>Purge flow</td>
<td>7.5 mL/min</td>
<td></td>
</tr>
<tr>
<td>Purge time</td>
<td>1.5 min</td>
<td></td>
</tr>
<tr>
<td>Parameter</td>
<td>Setting</td>
<td></td>
</tr>
<tr>
<td>----------------------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>Total flow</td>
<td>11.7 mL/min</td>
<td></td>
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<tr>
<td>Gas saver</td>
<td>On</td>
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<tr>
<td>Saver flow</td>
<td>20.0 mL/min</td>
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</tr>
<tr>
<td>Saver time</td>
<td>3.00 min</td>
<td></td>
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<tr>
<td>Gas type</td>
<td>Helium</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Column 1</th>
<th>Column 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary Column</td>
<td>Restek RTX18003 BAC 1</td>
</tr>
<tr>
<td></td>
<td>(not installed)</td>
</tr>
<tr>
<td>Max temperature</td>
<td>240 °C</td>
</tr>
<tr>
<td>Nominal length</td>
<td>30 m</td>
</tr>
<tr>
<td>Nominal diameter</td>
<td>320.00 um</td>
</tr>
<tr>
<td>Nominal film thickness</td>
<td>1.80 um</td>
</tr>
<tr>
<td>Mode</td>
<td>Ramped flow</td>
</tr>
<tr>
<td>Initial flow</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Initial time</td>
<td>4.50 min</td>
</tr>
<tr>
<td>Rate</td>
<td>Final flow</td>
</tr>
<tr>
<td></td>
<td>Final time</td>
</tr>
<tr>
<td>0.10</td>
<td>1.5</td>
</tr>
<tr>
<td>0.0(Off)</td>
<td>0.0</td>
</tr>
<tr>
<td>Post flow</td>
<td>2.5 mL/min</td>
</tr>
<tr>
<td>Nominal init pressure</td>
<td>1.69 psi</td>
</tr>
<tr>
<td>Average velocity</td>
<td>38 cm/sec</td>
</tr>
<tr>
<td>Inlet</td>
<td>Front Inlet</td>
</tr>
<tr>
<td>Outlet</td>
<td>MSD</td>
</tr>
<tr>
<td>Outlet pressure</td>
<td>vacuum</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FRONT DETECTOR (NO DET)</th>
<th>BACK DETECTOR (NO DET)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLUMN COMP 1</td>
<td>COLUMN COMP 2</td>
</tr>
<tr>
<td>(No Detectors Installed)</td>
<td>(No Detectors Installed)</td>
</tr>
</tbody>
</table>

**Thermal Aux 2**

**Use**: MSD Transfer Line Heater

**Description**

<table>
<thead>
<tr>
<th>Initial temp</th>
<th>260 °C (On)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial time</td>
<td>0.00 min</td>
</tr>
<tr>
<td># Rate</td>
<td>Final temp</td>
</tr>
<tr>
<td></td>
<td>Final time</td>
</tr>
<tr>
<td>1</td>
<td>0.0(Off)</td>
</tr>
</tbody>
</table>

**Post Run**

<table>
<thead>
<tr>
<th>Post Time</th>
<th>5.00 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oven Temperature</td>
<td>260 °C</td>
</tr>
<tr>
<td>Column 1 Flow</td>
<td>2.5 mL/min</td>
</tr>
</tbody>
</table>

**Time Table**

<table>
<thead>
<tr>
<th>Time</th>
<th>Specifier</th>
<th>Parameter &amp; Setpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>7673 Injector</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Front Injector</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample Washes</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
Sample Pumps | 2  
Injection Volume | 3.0 microliters  
Syringe Size | 10.0 microliters  
PreInj Solvent A Washes | 1  
PreInj Solvent A 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  
| Acquisition Mode | SIM  
| **MS Information** |  
| Solvent Delay | 1.30 min  
| EM Absolute | False  
| EM Offset | 0  
| Resulting EM Voltage | varies

**[SIM Parameters]**

**Group 1**

| Group ID | 1  
| Resolution | High  
| Plot 1 Ion | 84.0  
| Ions/Dwell In Group (Mass, Dwell) |  
| (84.0, 30) | (97.0, 120) | (98.0, 120) | (118.0, 120) | (119.0, 120) | (133.0, 60) | (162.0, 60) | (176.00, 120) | (191.00, 120) |
| (261.00, 120) |

**[MS Zones]**

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

**Timed Events**

**[Timed MS Detector Entries]**

| Time (min) | State (MS on/off) |
END OF MS ACQUISITION PARAMETERS

INSTRUMENT SETUP

An acceptable autotune must be obtained prior to batch analysis.

All autosampler syringe wash vials are filled with methanol.

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 MSNMDDYYx, where N is instrument number, MM = month, DD = day, YY= year and x = a letter indicating the batch being run, e.g. MS10410a001. 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., 2-13-2432 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 MSNMDDx001, where N = instrument number, MM = month, DD = day, and x = a letter indicating the batch being run, i.e. MS10410a001. 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. 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 i.e.:
   C:\MSDCHEM\1\DATA\MSNMMDDYy 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.: MSNMMDDYy001; 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 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? → 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\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. MS1041013a) 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.

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.

LOAD METHOD AND BATCH – FIRST LEVEL REVIEW

1. On the left side of the screen under the C drive, open C:\msdchem\1\ecm\retrieve
2. Under retrieve, click on the batch that was retrieved. When all files have been downloaded to the processing computer, verify that the appropriate method is present in the batch.
3. To load the method, right click on the method under the batch being processed 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 calibrator. 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, a
a single point calibrator 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 the 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 (level 1). Select Calibrate, Update One Level. When asked to requant files before update, select NO. Next update Calibration.

4. Select Update One Level, select replace responses and replace retention times and Replace Qualifier Ion Relative Responses, and choose the existing level ID (#1). 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. 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. On Update Calibration screen, select Update Level. Then click on Responses and Replace and on Retention Times and Replace. Then under Existing level ID, select the cal level to update and click on Do Update.

7. Requantitate the calibrator 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. 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. The blank must not contain detectable amounts of target analytes or significant interfering peaks.

9. 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.

10. 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.

11. 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.

12. Save files to ECM. Select ECM from the toolbar, select “Save multiple data files to ECM”. Select all files.

13. 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. Review the entire batch, using the same criteria as listed above. The controls must be within ± 20 % of the target value for blood, ± 30 % of the target value for non-blood matrices in addition to passing the integration, retention time, and peak shape criteria.

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. See REPORTING section.

2. Ion ratios must be within ± 20% of the average of the calibrators for blood samples. For tissues, the ion ratios must be within ± 30% of the average of the calibrators.

3. 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.

4. Make copies of all controls and the sequence list, enough to attach a set to each case in the batch.

5. 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. Evaluate and review data in accordance with guidelines in the Acceptance Criteria section. Annotate the results on the case file reporting sheet, and include the case chromatogram and control package in the case folder.
3. Nicotine and Cotinine are reported as detected or not detected.
4. Concentrations greater than or equal to 0.01 mg/L and meeting all other criteria are reported as detected. If the drug is less than 0.01 mg/l, not detected, or the criteria are not met, the drug is reported as “not detected”.
5. Compare your results to the results already reported for the case, to check if results are consistent with all other data. Notify supervisor if any inconsistencies are noticed.
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

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.
SPEware Corp Cerex Applications Manual.
System 48 Processor Users Guide.
Turbovap Users Guide.
Nicotine and Cotinine in Urine or Serum for gc or gcms confirmations using : 200 mg clean screen extraction column. Source –UCT Publication.
REVISION HISTORY

Ver 03.08.2013 1. Revision history implemented.
Ver 08.31.2015 1. Added internal standard acceptance criteria.
2. Defined the level of review(s)