

**OFFICE OF CHIEF MEDICAL EXAMINER  
CITY OF NEW YORK**

**METHADONE & EDDP  
BY  
LIQUID/LIQUID EXTRACTION  
AND  
GAS CHROMATOGRAPHY/MASS SPECTROMETRY  
(SELECTIVE ION MONITORING)**

**PRINCIPLE**

Methadone (Symoron, Dolophine, Amidone, Methadose, Physeptone, Heptadon and many other names) is a synthetic opioid developed in Germany in 1937. It is used medically as an analgesic and also as a maintenance anti-addictive and reductive preparation for use by patients with opioid dependence. Because it is structurally an acyclic analog of morphine, methadone also acts upon the same opioid receptors, and thus has many of the same effects. Because of its long duration of action, strong analgesic effects, methadone is also used in managing severe chronic pain. Eli Lilly and Company introduced methadone into the United States in 1947. The abuse of methadone results in approximately 5,000 overdose deaths per year in the United States.

Methadone is mainly used in the treatment of opioid dependence. It has cross-tolerance with other opioids including morphine, codeine and heroin, and offers very similar effects but with a longer duration. Oral doses of methadone can stabilize patients by mitigating opioid withdrawal syndrome or making it more tolerable. Higher doses can block the euphoric effects of heroin and morphine.

Methadone is produced and distributed by a number of pharmaceutical companies. The racemic hydrochloride is the only form available in most countries, such as the Netherlands, Belgium, France and in the United States (as of March 2008). The dextrorotary enantiomer of methadone, dextromethadone, is an NMDA antagonist rather than an opiate agonist. As a result, methadone medications used for opiate addiction occasionally will contain only levomethadone, the levorotary enantiomer. Levomethadone is available under the trade names Polamidone and Heptadon, among others.

In this procedure methadone (MTD) and its primary metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) are extracted from alkalized biological material following the addition of a deuterated internal standard. The compounds will be separated by gas chromatography and quantitated by operating a mass selective detector in the selected ion monitoring (SIM) mode.

**SAFETY**

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

## SPECIMEN PREPARATION

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

Blood	1.0 mL of the undiluted specimen
Urine	0.20 mL (1:5 dilution) for qualitative identification
Brain	1.0 mL of a 1:3 homogenate
Gastric Contents	1.0 mL of a 1:10 dilution
Liver	1.0 mL of a 1:5 homogenate
Vitreous Humor	1.0 mL of the undiluted specimen
Bile	1.0 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 <i>q.s.</i> to 20 mL of distilled water, or 2.0 g of a solid specimen homogenized with 18 mL of distilled water.
Urine	0.20 mL (1:5 dilution) for qualitative identification

**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 (AR) grade or higher. The chemical reagents required for the extraction procedure are prepared as indicated. In each case, the prepared reagent is stable for a minimum of six months. All new reagents are validated against the old ones prior to use.

1. **Deionized water**
2. **Buffer, pH 9.8**

Slowly add 212 g of Na<sub>2</sub>CO<sub>3</sub> to 2500 mL of distilled water in a 4000 mL Erlenmeyer flask. Once this is completely dissolved, slowly add 168 g of NaHCO<sub>3</sub>. Dilute to the mark with distilled water and mix on a stirring plate for one hour. Using a pH meter, verify that the pH is in fact 9.8. If the pH needs adjustment, use acid or base as needed to adjust to 9.8, document on the solution preparation sheet that the pH was checked. Date and initial preparation sheet.

3. **n-Butyl chloride (1-Chlorobutane)**
4. **0.5N HCl**

**CAUTION:** PREPARE THIS SOLUTION IN A FUME HOOD. USE APPROPRIATE SAFETY EQUIPMENT. ALWAYS ADD ACID TO WATER!

Slowly add 164 mL of concentrated HCl to 3500 mL of distilled water in a 4000 mL graduated cylinder. Dilute to the mark with distilled water and mix on a stirring plate for one hour.

5. **Sodium carbonate**

Slowly add 530 g of Na<sub>2</sub>CO<sub>3</sub> to 3000 mL of distilled water in a 4000 mL Erlenmeyer flask. Dilute to the mark with distilled water and mix on a stirring plate for one hour.

6. **Toluene/Heptane/Isoamyl Alcohol, 39:10:1(THI)**

Add 780 mL of toluene, 200 mL of heptane and 20 mL of isoamyl alcohol to a 1000 mL graduated cylinder. Briefly mix on a stirring plate, and then transfer to a 1 L repipeter bottle.

7. **Calibrators and controls**

Methadone (as free base) – Sigma Aldrich or equivalent

100 mg/L - 0.0100g into a 100 mL volumetric flask, QS with methanol

10 mg/L – 10 mL from 100 mg/L into a 100 mL volumetric flask, QS with methanol

EDDP HCl – Alltech or equivalent

100 mg/L - 0.0148g into a 100 mL volumetric flask. QS with methanol

10 mg/L – 10 mL from 100 mg/L into a 100 mL volumetric flask, QS with methanol

8. **Methadone-d9/ EDDP-d3 Internal Standard, 5 mg/L.**

Methadone-d9/ EDDP-d3 – Cerilliant or equivalent

5mg/L – 1 mL of 1.0 mg/mL methadone-d9 and 1 mL of 1.0 mg/mL of eddp-d3 into a 200 mL volumetric flask, QS with methanol.

## EXTRACTION PROCEDURE

1. Prepare a worklist of cases to be analyzed. Review with supervisor for any updates of priority (RUSH) cases or other special instructions. The batch size limit may be exceeded by authority of the section supervisor at need
2. Find and collect all samples on work list, and place in an empty specimen rack.
3. Obtain enough 16 x 150 mm screw-cap culture tubes (two per aliquot) and Teflon-lined caps to extract negative control, calibrators, controls and all requested case work. Label the tubes appropriately. Tubes should bear the entire toxicology number (e.g., 99-1234, **not** 1234), the specimen type and any appropriate dilution. If a sample matrix is urine then include a blank and controls using that matrix.
4. Obtain the required calibrators and controls for methadone and EDDP from the refrigerator and allow them to equilibrate to room temperature. After making note of the lot numbers on the lot form sheet, place these samples in random positions within the casework tubes. Prepare any required additional calibrators.
5. Pipette 1.0 mL of a matching, negative matrix into each of the blank and calibrator tubes (the negative matrix for urine samples is deionized water). Cover the tubes with a cap to

avoid possible contamination from other specimens. Fortify the tubes with various solutions as indicated.

Concentration (ng/mL)	$\mu$ L added	Calibrator solution
50	5	10 mg/L
100	10	10 mg/L
200	20	10 mg/L
500	5	100 mg/L
1000	10	100 mg/L
2000	20	100 mg/L
3000	30	100 mg/L

Concentration (ng/mL)	$\mu$ L added	Control solution
50	5	10 mg/L
1500	15	100 mg/L

6. After all calibrators and controls have been prepared, aliquot the case specimens. Mix each specimen container by gentle inversion two to four times and pipette 1.0 mL of each specimen into the appropriate tube. Make certain that the toxicology number on the specimen bottle exactly matches the number on the tube. Cap each tube after pipetting to prevent contamination from other cases. If a blood specimen is being pipetted and a clot is encountered, homogenize the specimen using the Polytron or a glass manual homogenizer, document on the blood bottle that it was homogenized and annotate on sequence list. Continue in this fashion until all cases have been pipetted.
7. Enter the date extracted in the Dataease database for each case picked up in the batch.

**Note: DO NOT WAIT UNTIL THE FOLLOWING DAY TO COMPLETE THE ABOVE!**

8. Spike, with syringe, 100  $\mu$ L of 5 mg/L d9-Methadone and d3-EDDP internal standard to all tubes.
9. Pipette 1.0 mL of the pH 9.8 buffer into each tube. Vortex each tube for five to ten seconds to thoroughly mix. Add 4.0 mL of n butyl chloride into each tube and cap all tubes. Briefly invert each tube to check for leaks. If a leak is found, transfer the tube contents to another appropriately labeled tube. Place all tubes on the mechanical shaker for 10 minutes using a low setting. Remove the tubes from the shaker and centrifuge for 10 minutes at  $\approx$  3000 rpm. If an emulsion is noticed, break up the emulsion by shaking or using a clean rod, and re-centrifuge. If the emulsion still persists, see the supervisor for alternate methods.
10. Transfer the upper, organic layer of each tube to a new appropriately labeled tube which contains 2.0 mL of 0.5N HCl. Make sure that the number on the two tubes match. Handle only one set of tubes at the same time. Cap and briefly invert each tube to check for leaks. Place on the mechanical shaker for 10 minutes on a low setting. Remove the tubes from the shaker and centrifuge for 10 minutes at  $\approx$  3000 rpm.

11. Upon removal from the centrifuge, uncap each tube and aspirate the upper, organic layer to waste using a suction/aspiration apparatus. Pipette 1.0 mL of sodium carbonate solution into the lower acid layer. Pipette 1.0 mL of the pH 9.8 buffer into each tube and vortex for five to ten seconds to mix.

**Note:** Addition of the sodium carbonate will cause release of CO<sub>2</sub> during the neutralization reaction which will result in foaming.

12. Add 100 µL of the toluene/heptane/isoamyl alcohol solution (THI) into each tube. Cap all tubes and place on the mechanical shaker for 10 minutes on a **high** setting. Remove the tubes from the shaker and centrifuge for 10 minutes at ≈ 3000 rpm. Using a Pasteur pipette, transfer the upper THI layer to a glass insert in an appropriately labeled vial.

**CAUTION:** DO NOT TRANSFER ANY OF THE AQUEOUS LAYER AT THIS STEP!

13. Transfer samples into screw cap vials and cap immediately. Transfer the vials to the GC/MS Instrumentation Laboratory and continue with analysis by gas chromatography/mass spectrometry.

## INSTRUMENTATION

Agilent MSD (6890 GC with 5973 Mass Spectrometer), Autosampler (Agilent 7683), and Agilent Chemstation with current revision of software. The method name for the methadone and EDDP assay is MSnMETHADEDDPSIM.M, where n = instrument number. Each MSD used for this assay will have a method with this name in the method directory.

### Top Level Parameters

#### 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 Post-Run Cmd/Macro
- Data Analysis Post-Run Cmd/Macro

### Instrumental Control Parameters

Sample Inlet: GC  
Injection Source: GC ALS  
Mass Spectrometer: Enabled

### 6890 GC Method

#### Oven

Initial Temp: 90 °C                      Maximum Temp: 320 °C  
Initial Time: 1.00 min                      Equilibration Time: 0.50 min

#### Ramps:

#	Rate	Final temp	Final Time
1	30.00	300	1.00
2	0 (off)		

Post Temp: 295 °C  
Post Time: 3.00 min  
Run Time: 9.00 min.

#### Front Inlet (SPLIT/SPLITLESS)

Mode: Splitless  
Initial Temp: 250 °C (On)  
Pressure: 16.24 psi (On)  
Purge Flow: 50.0 mL/min  
Purge Time: 1.0 min.  
Total Flow: 54.8 mL/min.  
Gas Saver: On  
Saver Flow: 20.0 mL/min  
Saver Time: 3.50 min.  
Gas Type: Helium

#### Back Inlet (SIM DIST)

Pressure: 4.00 psi (on)  
Gas Type: Helium

#### Column 1

Capillary Column  
Model Number: Restek 853563 Rxi  
50

#### Column 2

Capillary Column  
Model Number: Agilent 19091-  
60010 HP-101  
Methyl Siloxane

Max Temperature: 320 °C  
Nominal Length: 15.0 m  
Nominal Diameter: 250.0 µm

Nominal Film Thickness: 0.25 µm  
Mode: Ramped Flow  
Initial Flow: 2.0 mL/min.  
Initial Time: 6.00 min  
# Rate Final Flow Final Time  
1 1.00 3.5 8.25  
2 0.00 (off)  
Post flow: 4.00 mL/min  
Nominal init pressure: 16.24 psi  
Average Velocity: 48 cm/sec  
Inlet: Front Inlet  
Outlet: MSD  
Outlet Pressure: 4.00 psi

Max Temperature: 280 °C  
Nominal Length: 12.0 m  
Nominal Diameter: 200.00 µm

Nominal Film Thickness: 0.25 µm  
Mode: Constant pressure  
pressure: 4.00 psi  
Nominal init flow: 0.2 mL/min  
Average Velocity: 13 cm/sec

Inlet: Back inlet  
Outlet: (unspecified)  
Outlet Pressure: ambient

Front Detector (NPD)  
Temperature: 320 °C (On)  
Hydrogen Flow: 3.0 mL/min  
Air Flow: 60.0 mL/min  
Mode: Constant makeup flow  
Makeup flow: 29.0 mL/min  
Makeup gas type: Helium  
Adjust offset: 30.00  
Electrometer: On  
Read: On  
Equilibrium Time: 5.00

Back Detector (No det)

Signal 1  
Data Rate: 20 Hz  
Type: Test Plot  
Data Save: Off  
Zero: 0.0 (off)  
Range: 0  
Fast Peaks: Off  
Attenuation: 0

Signal 2  
Data Rate: 20 Hz  
Type: Test Plot  
Data Save: Off  
Zero: 0.0 (off)  
Range: 0  
Fast Peaks: Off  
Attenuation: 0

Column Comp 1  
(No Detectors Installed)

Column Comp 2  
(No Detectors Installed)

Thermal Aux 2  
Use: MSD Transfer line heater

Description:  
Initial Temp: 280 °C (On)

Initial Time:

#	Rate	Final temp	Final time
1	0.0 (off)		

POST RUN

Post time:	3.00 min
Oven Temp:	295 °C
Column 1 Flow:	4.0 mL/min
Column 2 Pressure:	0.0 PSI

TIME TABLE

Time	Specifier	Parameter & Setpoint
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GC Injector

Front Injector

Sample Washes	1
Sample Pumps	4
Injection Volume	3.00 µL
Syringe Size	10.0 µL
Pre Inj Solvent A Washes	1
Pre Inj Solvent B Washes	1
Post Inj Solvent A Washes	3
Post Inj Solvent B Washes	3
Viscosity Delay	0 Seconds
Plunger Speed	Fast
Pre Injection Dwell	0.00 Minutes
Post Injection Dwell	0.00 Minutes

Back Injector:

No parameters specified

Column 1 Inventory Number: 001

Column 2 Inventory Number: 002

MS Acquisition Parameters

General Information

Tune File	Atune.u
Acquisition Mode	SIM

MS Information

Solvent Delay:	5.50 minute
EMV Mode	Relative



Relative Voltage            0  
Resulting EM Voltage      Will depend upon Auto Tune Value

#### SIP Parameters

##### Group 1

Group ID:                    1  
Resolution:                 High  
Group Start Time:         0.00  
Plot 1 ion                    303.2  
Ions/Dwell in Group

Mass	Dwell	Mass	Dwell	Mass	Dwell
57.00	20	223.10	20	226.10	20
262.1	20	265.10	20	276.10	20
277.10	20	279.10	20	294.20	20
303.20	20				

#### MS Zones

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

### INSTRUMENT SETUP

A GCMS autotune must be performed at the start of each day of use.

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 re-login. 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 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 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 Level column, verify that the correct level numbers are entered for calibrators in this batch.
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.

**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\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 → 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**.

### ***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).
1. 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. Under Data File Directory verify that the data file path is C:\MSDCHEM\1\DATA\batch name.
  2. 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.
  3. 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 chain-of-custody verification of process steps to the printed sequence list.
  4. 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.
  5. 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.
  6. 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. Be sure to enter a copy of the sequence in the Sequence Logbook.

## **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 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 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 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 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 (50 ng/mL). Click OK. Repeat for remaining calibration levels (50, 100, 200, 500, 1000, 2000 and 3000 ng/mL). Select level 4 when prompted to update retention times.
4. Load the file associated with level 2 (100 ng/mL), 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. 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 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. The blank must not contain detectable amounts of target analytes or significant interfering peaks.
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. At the processing terminal, 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, checking that the ion peaks are present and integrated correctly (e.g., that the baseline is the most scientifically accurate one that can be drawn), that the ion ratios are  $\pm 20\%$  of the average of a calibrator, that the peaks are  $\pm 2\%$  of the calibrator retention times, and the peaks meet chromatography criteria. It is acknowledged that with some compounds the ion ratios will vary with concentration. As the target for ion ratios is set at the mid calibrator, for compounds at the lower or upper range of the calibration curve the acceptable ion ratios can be either  $\pm 30\%$  of the target value or  $\pm 20\%$  of the nearest calibrator provided that the peaks meet all other chromatographic criteria. The blank must not contain detectable amounts of target analytes or significant interfering peaks. The blood controls must be within  $\pm 20\%$  of the target value. For tissues, the controls are acceptable up to  $\pm 30\%$ .

**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.

2. 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.
3. Make copies of all controls, the  $r^2$  report and the sequence list, enough to attach a set to each case in the batch.

## **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 50 ng/mL are reported in ng/mL.
3. Concentrations lower than 50 ng/mL but meeting all other criteria are reported as less than 50 ng/mL. If the drugs are not detected or the reporting 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 it within quantitative curve limits.
5. Review other findings in the case, especially GCMS and GC results to see if they are consistent with these GCMS findings. If there are discrepancies, schedule additional testing to resolve them. If in doubt, consult with a supervisor.
6. 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.
7. 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.

SPEware Corp Cerex Applications Manual.

System 48 Processor Users Guide.

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## REVISION HISTORY

Ver 08.25.2014

1. Revision history implemented.
2. Analysis changed from GC/NPD to GC/MS SIM.

Ver 08.31.2015

1. Added internal standard acceptance criteria. Revised the "less than" reporting limit.
2. Defined the level of review(s)

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