

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

**COCAINE, ETHYLBENZOYLECGONINE, BENZOYLECGONINE,  
CODEINE, 6-MAM AND MORPHINE  
by  
SOLID PHASE EXTRACTION  
and  
GAS CHROMATOGRAPHY/MASS SPECTROMETRY  
(Selective Ion Monitoring)**

**PRINCIPLE**

Benzoyllecgonine (BE) is the major metabolite of cocaine and is the marker of cocaine use. Ethylbenzoyllecgonine (EBE) is a pharmacologically active substance that is formed when cocaine and ethanol are administered together. Morphine, as the major metabolite of heroin (diacetylmorphine), is an indication of possible heroin use. Morphine is also a drug with wide medicinal use. Codeine is a narcotic analgesic found in many pharmaceutical preparations alone or in combination with non-narcotic analgesics, antihistamines and other drugs. 6-monoacetyl morphine (6-MAM) is an intermediate in the metabolism of heroin to morphine and its presence in biological specimens indicates heroin use. Derivatization of these compounds to form silyl derivatives results in more suitable chromatographic properties. These derivatives are identified and quantitated by selected ion monitoring (SIM) gas chromatography/mass spectrometry (GCMS).

Drugs 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 and derivatized with MTBSTFA to form the t-butylsilyl derivatives. The derivatized samples are analyzed by GCMS.

This method is used as a backup procedure the analysis of these compounds, the primary being LCMS.

**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	1.0 mL of the undiluted specimen
Urine	1.0 mL for qualitative identification
Bile	1.0 mL of the undiluted specimen

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

### 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.

**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. **Sodium Fluoride** (Fisher Scientific - ACS Certified)
3. **Methanol** (Fisher Scientific - ACS Certified)
4. **Ammonium Hydroxide, NH<sub>4</sub>OH** (Fisher Scientific)

**Note:** Ammonium hydroxide will break down to ammonia and water and the ammonia will evaporate if the container is not kept closed. This will cause a pH decrease, making the reagent unsuitable for solid phase extraction. Use small lots of working solution (500 mL bottles), open the bottle only briefly to remove aliquots and recap immediately. If the solution appears old, discard and use a fresh bottle.

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

Dissolve 3.4 g Na<sub>2</sub>HPO<sub>4</sub> and 24.2 g NaH<sub>2</sub>PO<sub>4</sub> H<sub>2</sub>O in 1600 mL DI H<sub>2</sub>O.

Dilute to 2000 mL using DI H<sub>2</sub>O. Mix. Adjust pH to 6.0 + 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).

Store at 5 °C in glass.

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

#### 6. Hydrochloric Acid, 100 mM

Add 8.4 mL concentrated HCl to 800 mL Deionized water. Dilute to 1000 mL with deionized H<sub>2</sub>O.

Store at room temperature in glass or plastic.

Stability: 6 months. Inspect each day before use for contamination.

7. **Eluting solvent, CH<sub>2</sub>Cl<sub>2</sub> /IPA/NH<sub>4</sub>OH (78/20/2).** Prepare fresh each day of use.
8. **N,N-Dimethylformamide** (Sigma)
9. **MTBSTFA** (N-(tert-Butyldimethylsilyl)-N-methyltrifluoroacetamide) with 1% TBDMSCI, 97%, Aldrich Chemical, or equivalent.
10. **Morphine d<sub>3</sub>** (internal standard) , Cerilliant or equivalent, 1.0 mg/mL in 1 mL methanol, 99% pure (or equivalent).
11. **BE d<sub>3</sub>** (internal standard), Cerilliant International, 1.0 mg/mL in 1mL methanol, 99% pure, or equivalent.
12. **Cocaine d<sub>3</sub>** (internal standard), Cerilliant International, 1.0 mg/mL in 1mL acetonitrile, 99% pure, or equivalent.
13. **Morphine Cerilliant** or equivalent, 1.0 mg/mL in 1 mL methanol, 99% pure or equivalent.
14. **Codeine** Cerilliant or equivalent, 1.0 mg/mL in 1 mL methanol, 99% pure or equivalent.
15. **6-MAM** Cerilliant or equivalent, 1.0 mg/mL in 1 mL methanol, 99% pure or equivalent.
16. **BE** Cerilliant or equivalent, 1.0 mg/mL in 1 mL methanol, 99% pure or equivalent.
17. **Cocaine** Cerilliant or equivalent, 1.0 mg/mL in 1 mL acetonitrile, 99% pure or equivalent.
18. **EBE** Cerilliant or equivalent, 1.0 mg/mL in 1 mL methanol, 99% pure or equivalent.
19. **Morphine d<sub>3</sub>/BE d<sub>3</sub>/Cocaine d<sub>3</sub> working internal standard solution**
  - a. Pipet 1.0 mL of 1.0 mg/mL morphine d<sub>3</sub>, 1.0 mL of 1.0 mg/mL benzoylecgonine d<sub>3</sub>, 1.0 mL of 1.0 mg/mL cocaine d<sub>3</sub> into a 100 mL volumetric flask.
  - b. Q.S. to 100 mL with acetonitrile.
  - c. Transfer into properly labeled container – Morphine d<sub>3</sub>/BE d<sub>3</sub>/Cocaine d<sub>3</sub> (10 mg/L).

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

- d. Store at 4 °C.

20. **Cocaine/EBE/BE/Morphine/Codeine/6-MAM working calibrator solution (100 mg/L)**
  - a. Transfer 5 mL of 1 mg/mL primary standard solution to a 50 mL volumetric flask.
  - b. Q.S. to 50 mL with acetonitrile.
  - c. Transfer into a properly labeled container.

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

d. Store at 4°C.

**21. Cocaine/EBE/BE/Morphine/Codeine/6-MAM working calibrator solution (10 mg/L)**

- a. Transfer 5 mL of 100 mg/L calibrator solution to a 50 mL volumetric flask.
- b. Qs to 50 mL with acetonitrile.
- c. Transfer into properly labeled container.

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

d. Store at 4° C.

**22. Cocaine/EBE/BE/Morphine/Codeine/6-MAM working control solution (100 mg/L)**

- a. Transfer 5 mL of 1 mg/mL primary standard to a 50 mL volumetric flask.
- b. Q.S. to 50 mL with acetonitrile.
- c. Transfer to properly labeled container.

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

d. Store at 4° C.

**23. Cocaine/EBE/BE/Morphine/Codeine/6-MAM working control solution (10 mg/L)**

- a. Transfer 5 mL of 100 mg/L control solution to a 50 mL volumetric flask.
- b. Q.S. to 50 mL with acetonitrile.
- c. Transfer into properly labeled container.

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

d. Store at 4° C.

**24. QAS External Blood Control**

This is an unassayed blood control purchased through an external vendor with morphine, codeine, benzoylecgonine, EBE, and cocaine at a nominal concentration of 300 ng/mL. For each new lot of QAS control, the control is run at least 15 times, and the average of the values for each analyte becomes the new target value. The control range is  $\pm 20\%$  of the established target.

**25. Negative blood**

Calf or sheep blood obtained from outside source or equivalent. Sodium fluoride is added as a preservative. The preserved blood is validated as negative by in-house analysis. Store frozen (-10 °C or lower).

26. **Methylene Chloride CH<sub>2</sub>Cl<sub>2</sub>**
27. **2-Propanol C<sub>3</sub>H<sub>8</sub>O** (isopropanol, IPA)
28. **Polycrom Clin II Solid Phase Extraction Columns**, CEREX
29. **System 48 Processor** connected to nitrogen source.
30. **Waste Rack, SPE Rack, Collection Tube Rack**
31. **Evaporation Apparatus** (e.g., Turbovap, CEREX concentrator) connected to a nitrogen source.
32. **Gas Chromatography Column:**  
  
Restek RTX-5 SILMS crossbond 5% diphenyl -95% dimethyl polysiloxane. 30m x 0.25 mm ID x 0.25 µm DF, max temperature 360 °C. Model # 12723.

## EXTRACTION PROCEDURE

1. Aliquot 1 mL of validated negative matrix into each 16 x 125 mm tube labeled calibrator or control. Aliquot 1 mL of sample into each appropriately labeled 16 x 125 mm tube.

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

2. Five calibrators containing cocaine, EBE, BE, morphine, codeine, 6-MAM and IS are prepared as follows using the negative matrix:  
0.1mg/L - add 10 µL of 10 mg/L working calibrator solution  
0.5mg/L - add 50 µL of 10 mg/L working calibrator solution  
1.0mg/L - add 10 µL of 100 mg/L working calibrator solution  
1.5mg/L - add 15 µL of 100 mg/L working calibrator solution  
2.0mg/L - add 20 µL of 100 mg/L working calibrator solution
3. Three controls containing cocaine, EBE, BE, morphine, codeine, 6-MAM and IS are prepared as follows using the negative matrix:  
0.01mg/L - add 1 µL of 10 mg/L working control solution  
0.05mg/L - add 5 µL of 10 mg/L working control solution  
0.20mg/L - add 20 µL of 10 mg/L working control solution

For blood, an external control (QAS) is used with approximate values of 0.3 mg/L.

4. A matrix-matched blank must be included for each matrix in the batch.
5. Add 50 µL of 10 mg/L working internal standard solution to all tubes. The concentration of the internal standard in each sample is 0.5 mg/L.
6. Add 2 mL 100 mM phosphate buffer pH 6.0. Vortex for 30 seconds, then sonicate for 15 minutes.
7. Centrifuge sample for 10 minutes at 3000rpm.

8. Decant the supernatant into the Polychrom Clin II columns and apply nitrogen at a pressure of 2-4 psi.
9. Wash Column (All wash steps are pressurized at 2-4psi).
  - Pour 1 mL DI H<sub>2</sub>O onto column
  - Pour 1 mL 100 mM HCl onto column
  - Pour 1 mL CH<sub>3</sub>OH onto column
  - Pour 1 mL Ethyl Acetate onto column

10. Dry Column for 2 minutes at 25 psig

11. Prepare Elution Solvent

CH<sub>2</sub>Cl<sub>2</sub> /IPA/NH<sub>4</sub>OH (78/20/2) by mixing IPA/NH<sub>4</sub>OH, followed by CH<sub>2</sub>Cl<sub>2</sub>.

**Note:** Prepare elution solvent daily.

12. Elute Drugs

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

13. Dry under nitrogen at 40 °C to absolute dryness.

14. Derivatization

Add the following to dried conical tubes:

25 µL of N,N-dimethylformamide

50µL MTBSTFA

50 µL ethyl acetate

Cap tightly, vortex and incubate for 30 minutes at 80-90° C

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

16. Transfer derivatized 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.

17. Create batch sequence as specified in Instrument Setup.

18. Enter the date completed in the Dataase database, so the samples are not duplicated by another analyst.

**CAUTION:** DO NOT WAIT UNTIL THE FOLLOWING DAY TO DO THIS!

## INSTRUMENTATION

Agilent MSD (6890 GC with 5973 Mass Spectrometer), with Agilent 7673 or 7683 Autosampler, and Agilent Chemstation with current revision of software. The method name for this assay is

MSNOPBESIM.M, where N = instrument number. Each MSD used for Opiate/BE analysis will have a method with this name in the method directory.

The following ions are monitored for each drug:

Cocaine	82.1*, 182.1, 272.1, 303.1
Cocaine d <sub>3</sub>	85.1*, 185.1, 201.1
EBE	196.1, 272.1, 317.1
BE TBDMS	282.1, 403.3, 346.2
BE d <sub>3</sub> TBDMS	285.1, 349.2
Codeine TBDMS	313.1, 356.2, 178.1
6-MAM TBDMS	142.2, 441.3, 384.2
Morphine TBDMS	456.3, 513.3, 146.1
Morphine d <sub>3</sub> TBDMS	516.3, 459.3
* Alternate ions monitored	

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

Method Sections To Run:

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

Method Comments:

BE & OPIATE TBDMS DERIVATIVES - 30 meter RTX-5Sil MS column

**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	325 °C
--------------	-------------	--------------	--------

Initial time	4.00 min	Equilibration time	1.00 min
--------------	----------	--------------------	----------

<i>Ramps</i>			
#	Rate	Final temp	Final time
1	10.00	290	5.00
2	0.0(Off)		
Post temp	290 °C		
Post time	3.00 min		
Run time	24.00 min		

<i>Front Inlet (Unknown)</i>		<i>Back Inlet ()</i>
Mode	Pulsed Splitless	
Initial temp	250 °C (On)	
Pressure	15.5 psi (On)	
Pulse pressure	30.0 psi	
Pulse time	0.80 min	
Purge flow	7.7 mL/min	
Purge time	0.70 min	
Total flow	11.5 mL/min	
Gas saver	On	
Saver flow	20.0 mL/min	
Saver time	3.00 min	

<i>Column 1</i>		<i>Column 2</i>
Capillary Column		(not installed)
Model Number	RTX-5Sil MS	
Max temperature	325 °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	15.5 psi	
Average velocity	54 cm/sec	
Inlet	Front Inlet	
Outlet	MSD	
Outlet pressure	vacuum	

<i>FRONT DETECTOR (NO DET)</i>	<i>BACK DETECTOR (NO DET)</i>
COLUMN COMP 1	COLUMN COMP 2
(No Detectors Installed)	(No Detectors Installed)

<i>Thermal Aux 2</i>	
Use: MSD Transfer Line Heater	
<b>Description</b>	
Initial temp	280 °C (On)
Initial time	0.00 min
#	Rate Final temp Final time

1	0.0(Off)
---	----------

<i>Post Run</i>	
Post Time	3.00 min
Oven Temperature	290 °C
Column 1 Flow	1.2 mL/min

<i>Time Table</i>		
Time	Specifier	Parameter & Setpoint
7673 Injector		
<i>Front Injector:</i>		
Sample Washes	1	
Sample Pumps	2	
Injection Volume	3 µL	
Syringe Size	10 µL	
PreInj Solvent A Washes	2	
PreInj Solvent B Washes	2	
PostInj Solvent A Washes	2	
PostInj Solvent B Washes	2	
Viscosity Delay	0 seconds	
Plunger Speed	Fast	
PreInjection Dwell	0.00 minutes	
PostInjection Dwell	0.00 minutes	
<i>Back Injector:</i>		
No parameters specified		

### MS ACQUISITION PARAMETERS

<i>General Information</i>	
Tune File	Atune.u
Acquisition Mode	SIM
<i>MS Information</i>	
Solvent Delay	13.5 min
EM Absolute	False
EM Offset	0
Resulting EM Voltage	(Varies)

[Sim Parameters]

<i>Group 1 (for Cocaine, EBE and deuterated IS acquisition)</i>			
Group ID	1		
Resolution	High		
Plot 1 Ion	182.1		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(82.1, 30)	(85.1, 30)	(182.1, 30)
	(185.1, 30)	(196.1, 30)	(201.1, 30)
	(272.1, 30)	(303.1, 30)	(306.1, 30)

	(317.1, 30)		
--	-------------	--	--

<i>Group 2</i> (for BE and deuterated IS acquisition)			
Group ID	2		
Resolution	High		
Group Start time	16.5		
Plot 1 Ion	282.1		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(282.1, 30)	(285.1, 30)	(346.2, 30)
	(349.2, 30)	(403.3, 30)	

<i>Group 3</i> (for Codeine, 6-MAM, Morphine, and deuterated IS acquisition)			
Group ID	3		
Resolution	High		
Group Start time	18.0		
Plot 1 Ion	356.2		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(146.1, 30)	(178.1, 30)	(313.2, 30)
	(342.2, 30)	(356.2, 30)	(384.2, 30)
	(441.3, 30)	(456.3, 30)	(459.3, 30)
	(513.3, 30)	(516.3, 30)	

[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**

The GCMS must be autotuned each day, prior to batch analysis.

All autosampler syringe wash vials are filled with ethyl acetate.

Prepare a sequence using the following steps.

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

1. On the Method and Run toolbar, under **Sequence**, select **Load Sequence**. Select **default.s**. Click on Select.

2. In the Method and Run toolbar, under **Sequence**, select **Edit Sequence**. At the top of the screen under Data Path, click on Browse. Under **Select Data Path**, click on the **msdchem** folder. 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 correctness of pre-loaded entries. Starting at the first empty field, enter sample or QC information. For samples this would include aliquot number, laboratory number, specimen source, dilution if any (i.e., 2-11-2432 fem). If the sample has been diluted, enter the appropriate dilution factor in the Multiplier column. For QC samples use an appropriate designator. Enter the next sample in the batch in the next open field down the column.
4. In the Type column, select the corresponding sample type for each vial: Sample, Blank, Calibration or QC.
5. In the Vial column, click in the cell with the number 1, hold down the left mouse button and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column and Increment. Verify that the vial numbers are correct.
6. In the Method column, verify that the correct method is loaded in the first cell. Then click on the first cell containing the method name, hold down the left mouse and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column, No Increment. Verify that the method for each vial is correct.
7. In the Data File column, in the cell corresponding to the first vial, enter the data file name in the format MSNMMDDx001, where N = instrument number, MM = month, DD = day, and x = a letter indicating the batch being run, i.e. MS20411a001a. 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\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** or **No**.

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

*On the Sample Log Table:*

1. After inserting the DataPath keywords and Commands, Insert a Row.
  2. Select "**Keyword**" for "Type"
  3. Select "**MethodPath**" for "Method/Keyword"
  4. Check that the method for each sample is changed to the new method.
  5. Follow Steps 5-9 in the "**Setting-Up a Subsequence**" Section (see previous section).
13. On the Method and Run toolbar, under **Sequence**, select **Run Sequence**. In the dialog box under **Sequence Comment** enter the initials of the individual who has entered the sequence in the **Operator Field** (e.g., MPM). Under **Data File Directory** verify that the data file path is C:\MSDCHEM\1\DATA\batch name.
  14. Under **Sequence**, select **Save Sequence As**. Under File Name, type in the name of the folder that the batch will be saved to under DATA, MSNMMDDYYx (e.g. MS2041211a) Select **Save**. The extension ".s" will automatically be added.
  15. Under **Sequence** select **Print Sequence**. Verify that **Brief Format** is selected and click on **OK**. The sequence will be printed. Apply the preprinted labels for documenting verification of process steps to the printed sequence list.

16. Use the printed sequence list to load vials into the appropriate autosampler positions as indicated by the order on the printed sequence list. Check vial information against the sequence list and ensure that the vial is inserted in the correct numeric position in the autosampler as indicated on the sequence list. Document correct positioning by dating and initialing the appropriate line on the sequence list.
17. Under Sequence select **Run Sequence**. Verify that the Sequence comments and Data Field information are correct (i.e. verify that the proper sequence is loaded. If not, load the proper sequence). Click on **Run Sequence**.
18. After the batch is finished, unload the vials. Compare the vial information to the sequence list as they are removed, to verify that the correct vial was in the correct position. Date and initial the sequence list when this is completed. Annotate discrepancies if necessary

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

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

### **SAVE METHOD TO ECM**

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

### **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 (0.1mg/L). Click OK. Repeat for remaining calibration levels (0.5 1.0, 1.5 and 2.0 mg/L). Select level 3 when prompted to update retention times.
4. Load the file associated with level 3 (1.0 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. 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. 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. 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:** For non-blood matrix controls and case samples, the ion ratios may be accepted if they are within  $\pm 30\%$  of the average of the calibrators. This applies only to those cases where selected ion monitoring (SIM) is being used for quantitation and the analyte has been previously identified in the same specimen or confirmed in another specimen.

On rare occasions ion ratios will be out in exceptionally low or high concentrations. The operator must evaluate this and schedule proper dilutions or other analytical methodologies. Consult a supervisor 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.
4. Enter the sequence with autotune attached in the appropriate MS Sequence Logbook.

## **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,  $r^2$  table, and all controls appended.
2. Concentrations greater than 0.01 mg/L are reported in mg/L. Results are reported to two decimal points (e.g., 0.275 mg/L is reported as 0.27 mg/L).
3. Concentrations less than 0.01 mg/L are reported as "not detected".
4. Sample concentrations greater than the highest acceptable calibrator must be re-extracted with suitable dilution to bring it within the limits of the calibration curve, if a quantitative result is necessary.

5. Review other findings in the case, especially immunoassay and GC results to see if they are consistent with the GCMS findings. If there are discrepancies, schedule additional testing to resolve them. If in doubt, consult with a supervisor.
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**

Clarke'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.

Disposition of Toxic Drugs and Chemicals in Man, Ed. R.C. Baselt, Chemical Toxicology Institute, Foster City, CA, Fourth Edition, 1995.

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.

Turbovap. Users Guide.

## 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)

Uncontrolled Copy