

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

**FENTANYL
by
SOLID PHASE EXTRACTION
and
GAS CHROMATOGRAPHY/MASS SPECTROMETRY
(Selective Ion Monitoring)**

PRINCIPLE

Fentanyl is a synthetic narcotic analgesic of high potency and therefore found in biological specimens in low therapeutic concentrations and low toxic concentrations. Specimens in which fentanyl is detected by initial gas chromatography/mass spectrometry (GC/MS) screening procedure are quantitated by a selective ion monitoring (SIM) GC/MS method using deuterated fentanyl as the internal standard.

Fentanyl is 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. Quantitative analysis is performed by SIM GCMS using a five point calibration curve.

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	1.0 mL 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 for qualitative identification

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 ACS grade or better.

1. **Deionized water** (distilled can be substituted)
2. **Methanol** (Fisher Scientific - ACS certified, or equivalent)
3. **Ethyl Acetate** (Fisher Scientific - ACS certified, or equivalent)
4. **Ammonium Hydroxide, NH₄OH** (Fisher Scientific - ACS certified, or equivalent)
5. **Negative blood, serum, brain, liver**

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

6. **100 mM Sodium Acetate buffer (pH 4.5)**

Dissolve 11.72 g sodium acetate trihydrate in 1800 mL DI H₂O; add 6.5 mL glacial acetic acid. Dilute to 2000 mL using DI H₂O. Mix. Adjust pH to 4.5 ± 0.1 with sodium acetate or acetic acid.

Storage: room temperature in glass.

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

7. **pH 9.0 buffer**

Dissolve 40 g KHCO₃ in 1800 mL DI H₂O; add 20 g K₂CO₃. Dilute to 2000 mL using DI H₂O. Mix. Adjust pH to 9.0 ± 0.1 with KHCO₃ or K₂CO₃.

Storage: room temperature in glass.

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

8. **Polycrom Clin II Solid Phase Extraction Column**, CEREX, SPEware.
9. **System 48 Processor** connected to nitrogen source.
10. **Waste Rack, SPE Rack, Collection Tube Rack.**
11. **Evaporation apparatus** (e.g. Turbovap or SPEware) connected to a nitrogen source
12. **GCMS column**, Restek RTX-50 30M x 0.25 mm ID x 0.25 μ m DF, Model # 10523
13. **Fentanyl-d₅** (internal standard), Cerilliant or equivalent, 100 μ g/mL in 1 mL methanol, 99% pure, or equivalent.
14. **Fentanyl**, Cerilliant or equivalent, 1.0 mg/mL in 1 mL methanol, 99% pure, or equivalent.
15. **Fentanyl-d₅ working internal standard solution (1.0 mg/L)**
 1. Pipet 1.0 mL of Fentanyl-d₅ (100 μ g/mL) into a 100 mL volumetric flask.
 2. Q.S. to 100 mL with methanol.
 3. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.) Store at 2-4° C.
16. **Fentanyl working calibrator solution (1.0 mg/L)**
 1. Pipet 1.0 mL of 100 μ g/mL fentanyl calibrator solution into a 100 mL volumetric flask.
 2. Q.S. to 100 mL with methanol.
 3. Transfer into properly labeled container. (Include lot number, date prepared, initials of analyst, etc.) Store at 2-4° C.
17. **Fentanyl working calibrator solution (0.1 mg/L)**
 1. Pipet 10.0 mL of 1.0 mg/L working calibrator solution into a 100 mL volumetric flask.
 2. Q.S. to 100 mL with methanol.
 3. Transfer into properly labeled container. (Include lot number, date prepared, initials of analyst, etc.) Store at 2-4° C.
18. **Fentanyl working control solution (1.0 mg/L)**
 1. Pipet 1.0 mL of 100 μ g/mL fentanyl control solution into a 100 mL volumetric flask.
 2. Q.S. to 100 mL with methanol.
 3. Transfer into properly labeled container (include lot number, concentration, date prepared, initials of analyst, etc.). Store at 2-4° C.
19. **Fentanyl working control solution (0.1 mg/L)**
 1. Pipet 10.0 mL of 1.0 mg/L working control solution into a 100 mL volumetric flask.
 2. Q.S. to 100 mL with methanol.
 3. Transfer into properly labeled container (include lot number, concentration, date prepared, initials of analyst, etc.). Store at 2-4° C.

EXTRACTION PROCEDURE

1. Aliquot 1 mL of validated negative matrix into each 16 x 125 mm tube labeled calibrator(s) or control(s). 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 are prepared as follows using negative matrix:
 - 1 ng/mL – add 10 μ L of 0.1 mg/L fentanyl calibrator solution
 - 5 ng/mL – add 50 μ L of 0.1 mg/L fentanyl calibrator solution
 - 10 ng/mL – add 100 μ L of 0.1 mg/L fentanyl calibrator solution
 - 50 ng/mL – add 50 μ L of 1.0 mg/L fentanyl calibrator solution
 - 100 ng/mL – add 100 μ L of 1.0 mg/L fentanyl calibrator solution
3. Positive controls are prepared as follows using negative matrix:
 - 2 ng/mL – add 20 μ L of 0.1 mg/L fentanyl control solution
 - 10 ng/mL – add 100 μ L of 0.1 mg/L fentanyl control solution
4. A matrix matched blank must be included for each matrix type in the batch.
5. Add 50 μ L of fentanyl- d_5 internal standard solution (1.0 mg/L) to each tube.
6. Add 3 mL of pH 4.5 Sodium Acetate Buffer. Vortex for 30 seconds, then sonicate for 15 minutes.
7. Centrifuge at 3000 rpm for 10 minutes.
8. Decant the supernatant into the Polychrom Clin II column and apply nitrogen at a pressure of 2-4 psi.
9. Wash column (All wash steps are pressurized at 2-4 psi).
 - Pour 1mL pH 9.0 buffer onto column
 - Pour 1mL DI H₂O onto column
10. Dry columns for 20 minutes at 40 psi
11. Prepare Elution Solvent
 - Ethyl Acetate/NH₄OH (98/2). Mix well. Prepare fresh each day of use.
12. Elute Drugs
 - Place labeled 10 mL conical centrifuge tubes under each column to collect eluate. Elute with 2.0 mL.
13. Dry under nitrogen at 40°C to absolute dryness.
14. Reconstitute the dried extracts with 75 μ L of Ethyl Acetate.
15. Label autosampler vials indicating aliquot and toxicology number (ex: 1-YY-xxxx), specimen type, dilution, analyst, and date.
16. Transfer extract to an insert placed in a labeled autosampler vial. Immediately seal each vial with an aluminum seal using a crimper to avoid possible contamination from other samples. Samples may also be transferred into screw cap vials and capped immediately. When using aluminum seals, ensure that the seal is tight by attempting to rotate the seal. Reseal, using a new aluminum cap, if necessary. Do not wait until all transfers have been made to seal the vials. Samples are ready for MS injection.

17. Create batch sequence as specified in Instrument Setup.
18. 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-50 (Crossbond 100% methylphenyl polysiloxane) 30M x 0.25 mm ID x 0.25 µm DF, max temperature 320 °C. Model # 10523.

The following ions are monitored for each drug:

Fentanyl-d ₅	194, 250
Fentanyl	146, 189, 245

METHOD CONTROL PARAMETERS

Method Information for: C:\MSDCHEM\1\METHODS\MSXFENTANYL.M

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

Method Comments:

MS#X FENTANYL SIM METHOD

INSTRUMENT CONTROL PARAMETERS

Sample Inlet	GC
Injection Source	GC ALS
Mass Spectrometer	Enabled

HP6890 GC METHOD

OVEN

Initial temp	140 °C (On)	Maximum temp	320 °C
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Initial time	1.00 min	Equilibration time	0.50 min
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<i>Ramps</i>			
#	Rate	Final temp	Final time
1	15.00	295	3.50
2	0.0(Off)		
Post temp	295 °C		
Post time	5.00 min		
Run time	14.83 min		

<i>Front Inlet (Split/Splitless)</i>		<i>Back Inlet ()</i>
Mode	Pulsed Splitless	Not used
Initial temp	260 °C (On)	
Pressure	24.13 psi (On)	
Pulse Pressure	30.0 psi	
Pulse Time	0.80 min	
Purge flow	7.5 mL/min	
Purge time	1.5 min	
Total flow	11.2 mL/min	
Gas saver	On	
Saver flow	20.0 mL/min	
Saver time	2.00 min	
Gas type	Helium	

<i>Column 1</i>		<i>Column 2</i>
Capillary Column	Restek RTX50	(not installed)
Nominal length	30.0 m	
Nominal diameter	250.00 µm	
Nominal film thickness	0.25 µm	
Mode	Constant flow	
Initial flow	2.0 mL/min	
Nominal init pressure	24.13 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	
Description	
Initial temp	280 °C (On)
Initial time	0.00 min
# Rate Final temp Final time	

1	0.0(Off)
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<i>Post Run</i>	
Post Time	5.00 min
Oven Temperature	295 °C
Column 1 Flow	2.0 mL/min

<i>Time Table</i>		
Time	Specifier	Parameter & Setpoint
7673 Injector		
<i>Front Injector:</i>		
Sample Washes	1	
Sample Pumps	2	
Injection Volume	5.00 µL	
Syringe Size	10.0 µL	
PreInj Solvent A Washes	1	
PreInj Solvent B Washes	1	
PostInj Solvent A Washes	3	
PostInj Solvent B Washes	3	
Viscosity Delay	0 seconds	
Plunger Speed	Fast	
PreInjection Dwell	0.00 minutes	
PostInjection Dwell	0.00 minutes	
<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	12.0 min

[SIM Parameters]

<i>Group 1</i>			
Group ID	1		
Resolution	Low		
Group Start Time	12.00		
Plot 1 Ion	146.0		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(146.0, 125)	(189.0, 125)	(194.0, 75)
	(245.0, 100)	(250.0, 75)	

[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. Refer to the SOP "GCMS Tuning" for instructions.

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. Then click **1** and then click on the **Data** folder to highlight it. In lower left of dialog box, select **Make New Folder**. A folder with the name New Folder is created under DATA. Right click on **New Folder** and **Rename** or double click to highlight the folder name and change the entry. Rename the file using the format MSNMMDDYYx, where N is instrument number, MM = month, DD = day, YY= year and x = a letter indicating the batch being run, e.g. MS3040315a. Click **OK** which will take you back to the sample log table.
3. In the Sample column verify the correctness of pre-filled entries. Starting at the first empty row, enter sample or QC information. For samples, this would include aliquot number, laboratory number, specimen source, and dilution if any (i.e., 2-15-1234 fem). Enter the next sample in the batch in the next open field down the column.
4. In the Type column, select the corresponding sample type for each vial: Sample, Blank, Calibration.
5. In the Vial column, click in the cell with the number 1, hold down the left mouse button and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column and Increment. Verify that the vial numbers are correct including washes and reinjections.

6. In the Method column, verify that the correct method is loaded in the first cell. Then click on the first cell containing the method name, hold down the left mouse and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column, No Increment. Verify that the method for each vial is correct.
7. In the Data File column, in the cell corresponding to the first vial, enter the data file name in the format MSNMMDDx001, where N = instrument number, MM = month, DD = day, and x = a letter indicating the batch being run, i.e. MS30403a001. Click on this cell, hold down the left mouse and drag to the last vial number of the sequence (the cells will be highlighted). Right click and select Fill Column and Increment. Verify that the data file information for each vial is correct.
8. In the Comment column, enter any additional information for the vials (e.g. lot numbers)
9. In the Multiplier column, enter any sample dilutions.
10. In the **Level** column, verify that the correct level numbers are entered for calibrators in this batch.
11. Verify **No Update** is selected for all vials under **Update Rf** and **Update Rt**.
12. Review the information typed for the sequence. Correct any information as needed. Verify that the Data Path is C:\MSDCHEM\1\Data\current sequence name. Verify that the Method Path is C:\MSDCHEM\1\METHODS. Then Click **ok**.

Note: Occasionally, it will be desirable to run several subsequences in one batch. Use the instructions below to accomplish this.

Setting-Up a Subsequence

On the Sample Log Table:

1. Select "**Keyword**" for "Type"
2. Select "**DataPath**" for "Method/Keyword"
3. Under "Comment/KeywordString" type in the *new data path* for your subsequence i.e.: **C:\MSDCHEM\1\DATA\MSNMMDDYYy** where "N" is the instrument's number and "y" is the letter designated to the subsequence (**it must be different than that of the original sequence**).
4. The suffix of the data files must be different from that of the original; 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 2nd dialog box will pop-up: Create **C:\MSDCHEM\1\DATA\MSNMMDDYYy**? → 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).
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13. On the Method and Run toolbar, under **Sequence**, select **Run Sequence**. In the dialog box under **Sequence Comment** enter the initials of the individual who has entered the sequence in the **Operator Field** (e.g., MPM). Under **Data File Directory** verify that the data file path is C:\MSDCHEM\1\DATA\MSNMMDDYYx.
 14. Under **Sequence**, select **Save Sequence As**. Under File Name, type in the name of the folder that the batch will be saved to under DATA, MSNMMDDYYx (e.g. MS3040315a). Select **Save**. The extension ".s" will automatically be added.
 15. Under **Sequence** select **Print Sequence**. Verify that **Brief Format** is selected and click on **OK**. The sequence will be printed. Apply chain of custody labels to the printed sequence list.
 16. Use the printed sequence list to load vials into the appropriate autosampler positions. Check vial information against the sequence list and ensure that the vial is inserted in the correct numeric position in the autosampler. Date and initial the chain of custody label on the sequence printout, listing any comments, transfers, or exceptions.
 17. Under Sequence select **Run Sequence**. Verify that the Sequence comments and Data Field information are correct (i.e. verify that the proper sequence is loaded. If not, load the proper sequence). Click on **Run Sequence**.
 18. After the batch is finished, unload the vials. Compare the vial information to the sequence list as they are removed, to verify that the correct vial was in the correct position. Date and initial the sequence list when this is completed. Annotate discrepancies if necessary.

DATA TRANSFER AND PROCESSING - EXPORTING RAW DATA FOR PROCESSING

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

SAVE METHOD TO ECM

1. After the run finishes, the data files will be in the data subdirectory on the local chemstation and also automatically transferred to ECM. From the acquiring computer, make sure the proper method, the one used to acquire the data, is loaded. On the top toolbar under **ECM** click on **Save Method to ECM**.
2. Click on 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\”MSNMMDDYYx”

DATA REVIEW

There are three levels of review; the first level of review is the transference and processing of the raw data, this may be performed by any trained analyst; the second level of review is performed by an experienced analyst who is trained and signed off in data review, he / she will review the processed data; the third level of review is considered the final level of review, this can only be performed by the Laboratory Managers. He/she will review the data for the entire case ensuring that screening, confirmatory and quantitative analysis on the case have been completed and reported accurately. As needed, he/she will also schedule additional analysis and contact the Medical Examiner on the case to discuss any findings and / or review case history.

LOAD METHOD AND BATCH – FIRST LEVEL REVIEW

1. On the left side of the screen, open C:\msdchem\1\ecm\retrieve.
2. Under retrieve, click on the batch that was retrieved. Verify that the appropriate method is present in the batch folder.
3. To load the method, right click on the method and select load. This will bring up “Be sure changes are saved. Load now?” Click yes. If the method is not present, load the method by retrieving the method from ECM.
4. Click on any file in the batch to load it.

PERFORM BATCH CALIBRATION – PROCESSING – FIRST LEVEL REVIEW

Under enhanced data analysis:

1. Process the calibrators. Select Tools from the toolbar, DoLIST, and Quant, No Report (QT 1). Press Add, and OK. Select the files for this action to be performed on, in this case, calibrators only. Verify that the selected files are located in the correct subdirectory. Change the path if necessary. Click the → Arrow and Process.
2. Review the integrations of the targeted compounds for each calibrator; checking that the ion peaks are present and integrated correctly (i.e. the baseline is the most scientifically accurate one that can be drawn). Select View from the toolbar, QEDIT. Answer appropriately when prompted to save changes made to quantitation results when moving from file to file. Return to Data Analysis by selecting View from the toolbar, return to Data Analysis.
3. Update the existing calibration table (all levels). Select Calibrate, Update, Quick Levels Update. When prompted to clear responses, select YES. When asked to requant files before update, select NO. Select single data file/level option. Select the appropriate data file to associate with calibration level 1 (1 ng/mL). Click OK. Repeat for remaining calibration levels (5, 10, 50, 100 ng/mL). Select level 3 when prompted to update retention times.
4. Load the file associated with level 3 (10ng/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.
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. Verify multipliers/dilution factors are applied.
10. When review is complete, return to Data Analysis. Select report format by choosing Quantitate from the toolbar, Report Options. Check SIM style report and uncheck Internal Standards. Press OK.

11. To print reports, select Tools from the toolbar, DoLIST, Profile Quant w/o Calculations (QT 0,1,'P'), Add, and OK. Select files to print, click the → Arrow and Process.
12. Print the calibration table for the current batch by clicking **Calibrate** on the command line. Select **List, Calibrate Report** and click OK. The Calibration report will print to the screen. Review the r^2 values, then right click on the screen report to print it.
13. Save files to ECM. Select ECM from the toolbar, select "Save multiple data files to ECM". Select all files.
14. Save method to ECM. Select ECM from the toolbar, Save Method to ECM. Make sure data path is correct.

BATCH CLEAN UP

1. Select My Computer. Find the batch on the C drive at C:\msdchem\1\ecm\retrieve\batch. Right click on the batch to be deleted and select delete. Do not delete a batch that has not been successfully uploaded to ECM.

ACCEPTANCE CRITERIA

1. Acceptance range for calibrators is $\pm 20\%$ of the target concentration. Maximum two out of five calibrators may be dropped if outside of the acceptable range. However, the remaining acceptable calibrators must be re-processed and quantitative values for cases reported within the dynamic range of the acceptable calibration range.
2. Blood controls must be within $\pm 20\%$ of the target value and must pass integration, retention time ($\pm 2\%$ of the calibrator retention times), and peak shape criteria. The blank must not contain detectable amounts of target analytes or significant interfering peaks. For tissues, the controls are acceptable up to $\pm 30\%$ of the target value.
3. Ion ratios must be within $\pm 20\%$ of the target ion ratio, as determined by the midpoint calibrator, for blood samples. For non-blood samples, the ion ratios may be accepted within $\pm 30\%$, if the analyte has met acceptance criteria in a blood matrix.

Note: In some instances, ion 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.

4. The analyst shall choose from the standard levels a level at their discretion. All internal standard areas/peak heights for that sequence shall be compared to the internal standard(s) in that level. The acceptance criteria for the internal standard recovery/response shall be $\pm 30\%$ 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.

5. Make sufficient copies of the controls, calibration report, and the sequence list, enough to attach a set for each case in the batch.
6. Enter the sequence with tune attached in the sequence logbook for the appropriate MS.

SECOND LEVEL REVIEW

The Second Level Reviewer will review the processed data in its entirety according to the acceptance criteria section of this SOP.

REPORTING

After the batch has undergone second level review and has been printed, either the first level reviewer or the second level reviewer may report the data in the appropriate case file.

Reportusing 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. Fentanyl concentrations greater than or equal to 1 ng/mL are reported in ng/mL. Results are truncated and reported in whole numbers (i.e., 12.7 ng/mL is reported as 12 ng/mL),
4. Concentration lower than 1 ng/mL but meeting all other criteria is reported as “less than 1 ng/mL”. If the drug is not detected, or the criteria are not met, the drug is reported as “not detected”.
5. For qualitative analysis of urines, concentrations greater than 1 ng/mL are reported as “detected”.
6. Sample concentrations in excess of the highest calibrator must be re-extracted with suitable dilutions and with appropriate matrix matched controls to bring the concentration within the limits of the calibration curve.

7. If the positive blood matrix controls are $\pm 30\%$ of the target value, the samples may be reported qualitatively, as detected or not detected. If quantitative results are needed, consult a supervisor.
8. 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.
9. Submit the case file with all reports for Third Level Review.

THIRD LEVEL REVIEW (FINAL REVIEW)

The third and final level review can only be performed by the Laboratory Managers. He/she will review the data for the entire case according to all established criteria. They will ensure that screening, confirmatory and quantitative analysis on the case have been completed and reported accurately. As needed, they will also schedule additional analysis and contact the Medical Examiner on the case to discuss any findings and / or review case history.

REFERENCES

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Agilent 6890 GC System Installation Guide.

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Agilent 5973 & 5973 Network Mass Selective Detector Installation Guide.

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System 48 Processor Users Guide.

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REVISION HISTORY

Ver 03.08.2013	1. Revision History implemented
Ver 04.20.2015	1. Dilution control added.
Ver 08.31.2015	1. Added internal standard acceptance criteria. 2. Defined the level of review(s)

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