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.

REAGENTS AND MATERIALS

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

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

Specimens are diluted as follows:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Dilution</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1:3</td>
<td>5.0 g of brain homogenized with 10 mL of distilled water.</td>
</tr>
<tr>
<td>Liver</td>
<td>1:5</td>
<td>5.0 g of liver homogenized with 20 mL of distilled water.</td>
</tr>
<tr>
<td>Gastric</td>
<td>1:10</td>
<td>2.0 mL of liquid $q.s.$ to 20 mL of distilled water, or 2.0 g of a solid specimen homogenized with 18 mL of distilled water.</td>
</tr>
</tbody>
</table>

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

REAGENTS AND MATERIALS

All chemicals should be ACS grade or better.

1. Deionized water (distilled can be substituted)
2. Methanol (Fisher Scientific - ACS Certified)
3. Certified Negative Blood. Previously found to have no interfering peaks. Freeze validated negative blood. Discard after 3 months.
4. Certified Negative Liver. Previously found to have no interfering peaks. Freeze validated negative liver. Discard after 3 months.
5. Certified Negative Brain. Previously found to have no interfering peaks. Freeze validated negative brain. Discard after 3 months.
6. 100 mM Sodium Acetate buffer (pH 4.5)
   - Dissolve 5.86 g sodium acetate trihydrate in 800 mL DI H$_2$O; add 3.24 mL glacial acetic acid. Dilute to 1000 mL using DI H$_2$O. Mix. Adjust pH to 4.5 $\pm$ 0.1 with sodium acetate or acetic acid. Storage: room temperature in glass. Stability: 6 month. Inspect each day of use for contamination.
7. pH 9.0 buffer
   - Dissolve 40 g KHCO$_3$ in 1600 mL DI H$_2$O; add 20 g K$_2$CO$_3$. Dilute to 2000 mL using DI H$_2$O. Mix. Adjust pH to 9.0 $\pm$ 0.1 with KHCO$_3$ or K$_2$CO$_3$. Storage: room temperature in glass. Stability: 6 month. Inspect each day of use for contamination.
8. Polycrom Clin II Solid Phase Extraction Column, CEREX.
9. System 48 Processor connected to nitrogen source.
11. Evaporation apparatus (e.g. Turbovap or CEREX concentrator) connected to a nitrogen source.
12. Fentanyl-d5 (internal standard), Cerilliant or equivalent, 100 μg/mL in 1 mL methanol, 99% pure, or equivalent.

13. Fentanyl, Cerilliant or equivalent, 1.0 mg/mL in 1 mL methanol, 99% pure, or equivalent.

14. Fentanyl-d5 working internal standard solution (1.0 mg/L)
   1. Pipet 1.0 mL of fentanyl-d5 (100 μg/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.

15. Fentanyl working calibrator solution (1.0 mg/L)
   1. Pipet 1.0 mL of 100 μg/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.

16. 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.

17. Fentanyl working control solution (1.0 mg/L)
   1. Pipet 1.0 mL of 100 μg/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.

18. 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 (in each tube labeled as calibrator or in-house control) or sample into a 16 x 125 mm disposable culture tube labeled as to the contents.

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

2. Add appropriate amounts of standard solutions to negative matrix specimen tubes as follows:
   
   Five calibrators and appropriate matrix matched blank(s) (IS only) should be run with each batch of samples.

   Calibrators are prepared in the following concentrations:
   
   - 1 ng/mL – add 10 µL of 0.10 mg/L fentanyl calibrator solution
   - 5 ng/mL – add 50 µL of 0.10 mg/L fentanyl calibrator solution
   - 10 ng/mL – add 100 µL of 0.10 mg/L fentanyl calibrator solution
   - 50 ng/mL – add 50 µL of 1.0 mg/L fentanyl calibrator solution
   - 100 ng/mL – add 100 µL of 1.0 mg/L fentanyl calibrator solution

   Matrix matched controls are prepared as follows:
   
   - 2 ng/mL – add 20 µL of 0.10 mg/L fentanyl control solution
   - 10 ng/mL - add 100 µL of 0.10 mg/L fentanyl control solution

3. Add 50 µL of fentanyl-d₅ internal standard solution (1.0 mg/L) to each tube.

4. Add 3 mL of pH 4.5 Sodium Acetate Buffer. Vortex for 30 seconds, then sonicate for 15 minutes.

5. Centrifuge at 3000 rpm for 10 minutes.

6. Pour the supernatants into the appropriately labeled columns and apply nitrogen to achieve a pressure of 2-4 psi.

7. Wash column (All wash steps are pressurized at 2-4 psi).
   
   Pour 1mL pH 9.0 Buffer onto column
   Pour 1ml DI H₂O onto column

8. Dry columns for 15 minutes at 60 Psig

9. Prepare Elution Solvent
   
   Ethyl Acetate containing 2% NH₄OH. Mix well. Prepare fresh each day of use.

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

11. Dry under nitrogen at 40° C to **absolute** dryness.
12. Reconstitute the dried extracts with 75 μL of Ethyl Acetate.

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

14. Transfer extract to an insert placed in an autosampler vial. Immediately seal each vial with an aluminum seal using a crimper to avoid possible contamination from other samples. Samples may also be transferred into screw cap vials and capped immediately. When using aluminum seals, ensure that the seal is tight by attempting to rotate the seal. Reseal, using a new aluminum cap, if necessary. Do not wait until all transfers have been made to seal the vials. Samples are ready for MS injection.

15. Create batch sequence as specified in Instrument Setup.

16. 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

TOP LEVEL PARAMETERS

Method Information For: C:\HPCHEM1\METHODS\MSXFENTANYL.M

Method Sections To Run:

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

Method Comments:

FENTANYL METHOD – MSXFENTANYL.M

INSTRUMENT CONTROL PARAMETERS

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

HP6890 GC METHOD
OVEN

<table>
<thead>
<tr>
<th>Initial temp</th>
<th>140 °C (On)</th>
<th>Maximum temp</th>
<th>320 °C</th>
</tr>
</thead>
</table>

| Initial time | 1.00 min | Equilibration time | 0.00 min |

<p>| <strong>Ramps</strong> |  |
|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>#</th>
<th>Rate</th>
<th>Final temp</th>
<th>Final time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.00</td>
<td>295</td>
<td>8.0</td>
</tr>
<tr>
<td>2</td>
<td>0.0 (Off)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Post temp | 295 °C |
| Post time | 5.00 min |
| Run time | 19.33 min |

<table>
<thead>
<tr>
<th>Front Inlet (Unknown)</th>
<th>Back Inlet ()</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
<td>Pulsed Splitless</td>
</tr>
<tr>
<td>Initial temp</td>
<td>260 °C (On)</td>
</tr>
<tr>
<td>Pressure</td>
<td>24.14 psi (On)</td>
</tr>
<tr>
<td>Pulse Pressure</td>
<td>30.0 psi</td>
</tr>
<tr>
<td>Pulse Time</td>
<td>0.80 min</td>
</tr>
<tr>
<td>Purge flow</td>
<td>7.5 mL/min</td>
</tr>
<tr>
<td>Purge time</td>
<td>1.5 min</td>
</tr>
<tr>
<td>Total flow</td>
<td>11.2 mL/min</td>
</tr>
<tr>
<td>Gas saver</td>
<td>On</td>
</tr>
<tr>
<td>Saver flow</td>
<td>20.0 mL/min</td>
</tr>
<tr>
<td>Saver time</td>
<td>2.00 min</td>
</tr>
<tr>
<td>Gas type</td>
<td>Helium</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Column 1</th>
<th>Column 2 (not installed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary Column</td>
<td>Restek RTX50</td>
</tr>
<tr>
<td>Nominal length</td>
<td>30 m</td>
</tr>
<tr>
<td>Nominal diameter</td>
<td>250.00 um</td>
</tr>
<tr>
<td>Nominal film thickness</td>
<td>0.25 um</td>
</tr>
<tr>
<td>Mode</td>
<td>Constant flow</td>
</tr>
<tr>
<td>Initial flow</td>
<td>2.0 mL/min</td>
</tr>
<tr>
<td>Nominal init pressure</td>
<td>24.15 psi</td>
</tr>
<tr>
<td>Average velocity</td>
<td>54 cm/sec</td>
</tr>
<tr>
<td>Inlet</td>
<td>Front Inlet</td>
</tr>
<tr>
<td>Outlet</td>
<td>MSD</td>
</tr>
<tr>
<td>Outlet pressure</td>
<td>vacuum</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FRONT DETECTOR (NO DET)</th>
<th>BACK DETECTOR (NO DET)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLUMN COMP 1</td>
<td>COLUMN COMP 2</td>
</tr>
<tr>
<td>(No Detectors Installed)</td>
<td>(No Detectors Installed)</td>
</tr>
</tbody>
</table>
Thermal Aux 2
Use: MSD Transfer Line Heater

**Description**
- Initial temp: 280 °C (On)
- Initial time: 0.00 min
- # Rate Final temp Final time
- 1 0.0(Off)

**Post Run**
- Post Time: 5.00 min
- Oven Temperature: 295 °C
- Column 1 Flow: 2.0 mL/min

**Time Table**

<table>
<thead>
<tr>
<th>Time</th>
<th>Specifier</th>
<th>Parameter &amp; Setpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>7673 Injector</td>
<td>Front Injector:</td>
<td></td>
</tr>
<tr>
<td>Sample Washes</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sample Pumps</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Injection Volume</td>
<td>3.0</td>
<td>microliters</td>
</tr>
<tr>
<td>Syringe Size</td>
<td>10.0</td>
<td>microliters</td>
</tr>
<tr>
<td>PostInj Solvent A Washes</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>PostInj Solvent B Washes</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Viscosity Delay</td>
<td>0</td>
<td>seconds</td>
</tr>
<tr>
<td>Plunger Speed</td>
<td>Fast</td>
<td></td>
</tr>
<tr>
<td>PreInjection Dwell</td>
<td>0.00</td>
<td>minutes</td>
</tr>
<tr>
<td>PostInjection Dwell</td>
<td>0.00</td>
<td>minutes</td>
</tr>
<tr>
<td>Back Injector:</td>
<td>No parameters specified</td>
<td></td>
</tr>
</tbody>
</table>

**MS ACQUISITION PARAMETERS**

**General Information**
- Tune File: ATUNE.U
- Acquisition Mode: SIM

**MS Information**
- Solvent Delay: 12.0 min
- EM Absolute: False
- EM Offset: 0
- Resulting EM Voltage: varies

**[SIM Parameters]**

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group ID</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>High</td>
</tr>
</tbody>
</table>
Group Start Time | 12.00
---|---
Plot 1 Ion | 146.0
Ions/Dwell In Group | (Mass, Dwell)
| (Mass, Dwell)
| (Mass, Dwell)
| (146.0, 125) | (151.0, 75) | (189.0, 125)
| (194.0, 75) | (245.0, 100) | (250.1, 75)

[MS Zones]

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

Timed Events

[Timed MS Detector Entries]

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>State (MS on/off)</th>
</tr>
</thead>
</table>

END OF MS ACQUISITION PARAMETERS

INSTRUMENT SETUP

An acceptable autotune must be obtained prior to batch analysis.

All autosampler syringe wash vials are filled with methanol.

Prepare a sequence using the following steps.

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

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

2. In the Method and Run toolbar, under Sequence, select Edit Sequence. At the top of the screen under Data Path, click on Browse. Under Select Data Path, click on the msdchem folder. The click 1 and then click on the Data folder to highlight it. In lower left of dialog box, select Make New Folder. A folder with the name New Folder is created under DATA. Right click on New Folder and Rename or double click to highlight the folder name and change the entry. Rename the file using the format MSNMMDYYx, 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 MSNNMDDx001, 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\MSNNMDDYYx where “N” is the instrument’s number and “x” is
the letter designated to the subsequence (it must be different than that of the original sequence).

4. The suffix of the data files must be different from that of the original; i.e.: MSNMMDDyy001; the subsequence data files must start with 1 again.

5. After typing in the entire sequence, save sequence accordingly.

6. Go to Sequence → Simulate Sequence → Run Sequence.

7. A dialog box will pop-up: DataPath C:\MSDCHEM\1\DATA\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).

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**

All processing and review are 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**

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"

**LOAD METHOD AND BATCH**

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

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 (1.0 ng/mL). Click OK. Repeat for remaining calibration levels (5.0, 10.0, 50.0, 100 ng/mL). Select level 3 when prompted to update retention times.

4. Load the file associated with level 3 (10.0 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. Select my computer. Find the batch on the C drive at C:\msdchem\1\ecm\retrieve\batch. Right click on the batch to be deleted and select delete. Do not delete a batch that has not been successfully uploaded to ECM.

**ACCEPTANCE CRITERIA**

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

   **Note:** Sometimes ratios will be off in exceptionally low or high concentrations. The operator must evaluate this and schedule proper dilutions or other methodologies, as needed. See **REPORTING** section.

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

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

4. Enter the sequence with tune attached in the sequence logbook for the appropriate MS.
REPORTING

After the batch has been reviewed and printed, it must be reported, using the following guidelines:

1. Each case printout must have a copy of the sequence and all controls appended.
2. Evaluate and review data in accordance with guidelines in the Acceptance Criteria section. Annotate the results on the case file reporting sheet, and include the case chromatogram and control package in the case folder.
3. Fentanyl concentrations are reported to one decimal point in units of ng/mL or ng/g (i.e., 12.7 ng/ml is reported as 12 ng/mL).
4. Concentrations lower than 1 ng/mL but meeting all other criteria are 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. 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.
6. Compare results to the results already reported for the case to check if results are consistent with all other data. Notify supervisor if any inconsistencies are noticed.

REFERENCES

Agilent 6890 GC System Installation Guide.
Agilent 6890 GC System Users Guide.
Agilent 6890 GC System Standard Operating Procedures.
Agilent 5973 & 5973 Network Mass Selective Detector Installation Guide.
Agilent 5973 & 5973 Network Mass Selective Detector Users Guide.
SPEware Corp Cerex Applications Manual.
System 48 Processor Users Guide.
Turbovap Users Guide.