

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

**METOCLOPRAMIDE, TRIMETHOPRIM, TICLOPIDINE, TRAMADOL, TRAMADOL
METABOLITES (O-DESMETHYL AND N-DESMETHYL TRAMADOL),
BROMAZEPAM, VENLAFAXINE AND O-DESMETHYL VENLAFAXINE
BY
SPE AND GC-NPD ANALYSIS**

PRINCIPLE

Solid phase extraction (SPE) is used to extract the above mentioned compounds due to poor recovery by liquid-liquid extraction. Drugs are temporarily bound to a sorbent in the solid phase column as the prepared sample is poured through these columns. The columns are washed to remove interfering compounds, followed by elution of drugs. The eluent is evaporated and then reconstituted. The resulting solution is analyzed by GC-NPD. Quantitative analysis is performed using a four-point calibration curve and internal standard.

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	2.0 mL of undiluted specimen
Urine	2.0 mL of undiluted specimen
Brain	2.0 mL of a 1:3 homogenate
Gastric Contents	2.0 mL of a 1:10 dilution
Liver	2.0 mL of a 1:5 homogenate
Vitreous Humor	2.0 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 deionized water.
Liver 1:5	5.0 g of liver homogenized with 20 mL of deionized water.
Gastric 1:10	2.0 mL of liquid <i>q.s.</i> to 20 mL of deionized water, or 2.0 g of a solid specimen homogenized with 18 mL of deionized water.

Note: Do not use homogenates older than two weeks unless low sample size requires it. Discuss with supervisor and note in case record.

Note: In preparing gastric homogenates, the entire submitted amount of gastric contents must be homogenized prior to sampling.

REAGENTS AND MATERIALS

All chemicals should be analytical reagent grade or better.

1. Deionized water (DI H₂O)

2. Negative Matrices

Certified negative matrices (blood, brain, and liver from calf or sheep) are obtained from outside source and validated in-house. These are stored frozen (-10 ° C or lower).

3. Sodium acetate trihydrate. Certified ACS. Fisher Scientific or equivalent.

100mM sodium acetate buffer (pH 4.5)

Dissolve 2.93 g sodium acetate trihydrate in 400 mL DI H₂O; add 1.62mL glacial acetic acid. Dilute to 500 mL using DI H₂O. Mix. Adjust pH to 4.5 ± 0.1 with sodium acetate or acetic acid.

Storage: in glass; room temperature.

Stability: 6 months; inspect daily before use for contamination.

4. pH 9.0 buffer

Dissolve 20 g KHCO₃ in 800 mL DI H₂O; add 10 g K₂CO₃. Dilute to 1L using DI H₂O. Mix. Adjust pH to 9.0 ± 0.1 with KHCO₃ to lower the pH, or K₂CO₃ to raise the pH.

Storage: room temperature in glass.

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

5. HCl, concentrated, 12.1N. Fisher Scientific or equivalent.

Hydrochloric Acid, 100mM

Add 8.4mL concentrated HCl to 800 mL Deionized water. Dilute to 1000 mL with deionized H₂O.

Store at room temperature in glass or plastic.

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

6. **Methanol** (Fisher Scientific - ACS Certified)
7. **Ethyl Acetate**, Fisher Scientific Optima or equivalent.
8. **Ammonium Hydroxide, NH₄OH** (Fisher Scientific)

Eluting solvent: Ethyl acetate/NH₄OH (98/2). Prepare fresh daily for use.

Note: Ammonium hydroxide will break down to ammonia and water. 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 (3 mL bottles). If the solution appears old, discard and use a fresh bottle. Always keep ammonium hydroxide refrigerated.

9. Preparation calibrators and controls

The following stock solutions are prepared to yield a final concentration of 100 mg/L:

<u>Drug (manufacturer, or equivalent)</u>	<u>Amount</u>	<u>Total Volume</u>	<u>Solvent Used</u>
Metoclopramide HCl (Sigma Aldrich)	0.0059 g	50 mL	Methanol
Trimethoprim (Sigma Aldrich)	0.0050 g	50 mL	Methanol
Ticlopidine HCl(Sigma Aldrich)	0.0057 g	50 mL	Methanol
Tramadol HCl(Pharm Res)	0.0056 g	50 mL	Methanol
O-desmethyl tramadol (Cerilliant)	1mL from 1mg/mL stock	10 mL	Methanol
N-desmethyl tramadol (Cerilliant)	1mL from 1mg/mL stock	10 mL	Methanol
Venlafaxine (Cerilliant)	1mL from 1mg/mL stock	10 mL	Methanol
O-desmethyl venlafaxine HCl (Wyeth Ayerst)	0.0057 g	50 mL	Methanol
Bromazepam (Sigma Aldrich)	0.0025 g	25 mL	Methanol

10. **Polycrom Clin II Solid Phase Extraction Columns**, CEREX
11. **System 48 (or CEREX 48) Processor** connected to nitrogen source.
12. **Waste Rack, SPE Rack, Collection Tube Rack.**
13. **Concentrator (Turbovap or CEREX)** connected to a nitrogen source.
14. **Toluene/Heptane/Isoamyl Alcohol, 39:10:1(THI)**

Add 780 mL of toluene, 200 mL of heptane and 20 mL of isoamyl alcohol to a 1000 mL graduated cylinder. Briefly swirl by hand, then transfer to a 1L repipeter.

EXTRACTION PROCEDURE

Calibrators and controls are prepared by adding validated stock solutions to 2.0 mL certified negative matrix in appropriately labeled 16 x 125 mm tubes. A four-point calibrator curve is prepared consisting of 0.05 mg/L, 0.2 mg/L, 1.0 mg/L, and 3.0 mg/L. Controls are also prepared at 0.025 mg/L, 0.5 mg/L, and 1.0 mg/L.

1. **Calibrators:** Using the 100mg/L validated calibrator stock solution add the following amounts to the appropriately labeled test tube

Final Concentration (mg/L)	Stock Solution Concentration (mg/L)	Spiking Volume (µL)
0.05	100	1
0.2	100	4
1.0	100	20
3.0	100	60

2. **Controls:** Using the 100 mg/L validated control stock solution add the following amounts to the appropriately labeled test tube

Final Concentration (mg/L)	Stock Solution Concentration (mg/L)	Spiking Volume (µL)
0.025	100	0.5
0.5	100	10
1.0	100	20

3. After all calibrators and controls have been prepared, aliquot the case specimens. Make certain that the toxicology number on the specimen bottle exactly matches the number on the tube. Homogenize specimen if needed. Mix each room temperature specimen container by gentle inversion a couple of times and pipet 2.0 mL of each specimen into the appropriately labeled 16 x 125 mm culture test tube. After sampling all the cases, return cases back to storage.
4. Add 40 µL of methapyrilene internal standard (50 mg/L) to all tubes (blank, calibrators, controls, and cases) then vortex.
5. Add 6mL 100 mM sodium acetate buffer pH 4.5 to the tubes. Mix by Vortex for 30 seconds then sonicate for 15 minutes. Centrifuge sample for 15 minutes at \approx 3000 rpm.
6. Decant the supernatant into the appropriately labeled Polychrom Clin II column and apply nitrogen at a pressure of 2-4 psi.
7. Prepare the eluting solvent by pouring 98 mL of Ethyl Acetate into a glass flask with stopper; add 2 mL of refrigerated NH_4OH . Place flask on stir plate and allow eluting solvent to mix while completing the remaining steps of the extraction.

Note: Prepare fresh eluting solvent each day of use.

8. Wash Column (all wash steps are pressurized at 2-4psi).
Pour 2 mL pH 9.0 buffer onto column.
Pour 2 mL 100 mM HCl onto column.
Pour 2 mL CH₃OH onto column.
Pour 2 mL ethyl acetate onto column.
Dry column for 1 minute at \approx 25 psi (Max Flow)
9. Elute Drugs
Place labeled 10 mL conical centrifuge under each column to collect eluate. Elute with 2mL eluting solvent (ethyl acetate/NH₄OH, 98/2); collect eluent at 1 to 2mL/minute. Apply pressure as necessary to achieve the desired flow rate.
10. Dry under nitrogen at room temperature to absolute dryness using a concentrator.
11. Reconstitute with 100 μ L of TH1. Mix by vortex and transfer to the glass insert in the appropriately labeled autosampler vial.
12. Run on GC-NPD by using the GC quantitation method; follow the "Basic Drugs Quantitation by Gas Chromatography" SOP for method and instrument set-up.
13. Process, review and report sequence using the same criteria specified in the "Basic Drugs Quantitation by Gas Chromatography" SOP.

INSTRUMENTATION

The instrumentations used for analysis is the Agilent 6890 gas chromatograph equipped with a 7683 Series autosampler and a Agilent 6890 Series nitrogen-phosphorous detector. A computer with Agilent Chemstation software is used to gather the data and Chemstation Enhanced Data Analysis software is used to process the data. A selection of three different capillary columns is available for analyses.

COLUMNS

The primary column used in the GC section is a 10.0 m x 0.53 mm x 2 μ m HP-17 (50% phenyl methylsiloxane) megabore capillary column. This column is used for most routine work, both qualitative and quantitative.

The secondary column used in the GC section is a 10.0 m x 0.53 mm x 2.65 μ m HP-5 (5 % phenyl methylsiloxane) megabore capillary column. This column is used to analyze compounds that co-elute on HP-17, or are excessively retained on HP-17.

The third column used is the Restek (RTX50) (Crossbonded 100% methyl phenyl polysiloxane) column (15m x 0.25mm x 0.25 μ m film thickness). This column is used as needed to analyze compounds that co-elute.

INSTRUMENT SETUP

6890 Series II Gas Chromatogram

1. Ensure that the selected GC is operational and not in need of repair. If maintenance is required, consult the appropriate manual and notify the supervisor.
2. Make sure GC power on.
3. Check that the NPD bead voltage is set to current value as listed on the instrument.
4. The Chemstation method file for quantitation is usually named GCxnQNT.M, where the x refers to the instrument number. There should be a method file with the appropriate name in each method subdirectory. (When using RTX 50, method name is GC4RQNT.M.) Load the appropriate method for the analysis. Consult supervisor if correct method is unclear.
5. On Chemstation verify that all other GC parameters are set correctly; go to Method, Edit Entire Method. Wait for "Ready".

Method Section to Edit:

- (X) Method Information
- (X) Instrument/Acquisition
- Method Information
- (X) Save copy of method with data
- (X) Data Acquisition
- () Data analysis

Inlet and Injection Parameters

Sample Inlet: GC
Injection Source: GC ALS

GC Injector (7683 Series Injector)

Select Single or Dual

Injector Parameters (Front or Back or Both)

Sample Washes	1
Sample Pumps	4
Injection Volume	5.0 microliters
Syringe Size	10.0 microliters
PreInj Solvent A Washes	0
PreInj Solvent B Washes	0
PostInj Solvent A Washes	5
PostInj Solvent A Washes	5
Viscosity Delay	0 seconds
Plunger Speed	Fast
PreInjection Dwell	0 minutes
PostInjection Dwell	0 minutes

Inlet Parameters

<i>Front Inlet</i>	<i>(Split/Splitless)</i>	<i>Back Inlet</i>	<i>(Split/Splitless)</i>
Mode	Pulsed Splitless	Mode	Pulsed Splitless
Initial Temp	275 °C (on)	Initial Temp	275 °C (on)
Pressure	5.20 psi (on)	Pressure	5.20 psi (on)
Pulse pressure	10.0 psi	Pulse pressure	10.0 psi
Pulse time	0.60 min	Pulse time	0.60 min
Purge flow	30.0 mL/min	Purge flow	30.0 mL/min
Purge time	2.00 min	Purge time	2.00 min
Total Flow	42.1 mL/min	Total Flow	42.1 mL/min
Gas Saver	off	Gas Saver	Off
Gas type	Helium	Gas type	Helium

Column Parameters

Capillary column			
Model number			19095L-121
HP-50+ 50% Phenyl Methyl Siloxane			
Max Temp			310 °C
Nominal length			10.0m
Nominal diameter			530.00 um
Nominal film thickness			2.00um
Mode			Ramped pressure
Initial Pressure			5.20 psi
Initial Time			12.00 min
#	Rate	Final Pressure	Final time
1	3.20 psi/min	8.2 psi	0.0
2		0.0 (off)	
Post pressure		5.20 psi	
Nominal init pressure		5.20 psi	
Nominal init flow		15.4 ml/min	
Average velocity		131 cm/sec	
Inlet		Front, Back, or Both	
Outlet (detector)		Front, Back, or Both	
Outlet pressure		ambient	

Oven Parameters (6890)

Initial temp: 120 °C
Initial time: 2.00 min

Maximum temp: 280 °C
Equilibration time: 1.00 min

Ramps:

#	Rate (C/min.)	Final Temp. (°C)	Final Time (min.)
1	15.0	280	18.00
2	0.0 (off)		
Post temp.	120 °C		
Post time	1.00 min		
Run time	30.67 min		

Detector Parameters

Front detector (NPD)		Rear detector(NPD)	
Temperature	325 °C mL/min(on)	Temperature	325 °C (on)
Hydrogen flow	3.0 mL/min (on)	Hydrogen flow	3.0 mL/min (on)
Air flow	60.0 mL/min (on)	Air flow	60.0 mL/min (on)
Mode	Constant column+makeup flow	Mode	Constant column+makeup flow
Combined flow	30.0 mL/min	Combined flow	30.0 mL/min
Makeup flow	On	Makeup flow	On
Makeup gas type	Helium	Makeup gas type	Helium
Adjust offset	30.00	Adjust offset	30.00
Electrometer	On	Electrometer	On
Bead	On	Bead	On
Equilibration time	0.00	Equilibration time	0.00

Signals

(Select Front, Back, or Both)

Signal 1		Signal 2	
Data rate	20 Hz	Data rate	20 Hz
Type	Front detector	Type	Rear detector
Save Data	Partial	Save Data	Partial
Start Time	1.0 min	Start Time	1.0 min
Stop	30 min		30 min

HP-5 COLUMN (Column 2)

Capillary column	
Model number	HP 19095J-121
HP-5 5% Phenyl Methyl Siloxane	
Max Temp	300 °C

Nominal length		10.0m	
Nominal diameter		530.00 um	
Nominal film thickness		2.65um	
Mode		Ramped pressure	
Initial Pressure		5.20 psi	
Initial Time		12.00 min	
#	Rate	Final Pressure	Final time
1	3.20	8.20	0.00
2	0.0 (off)		
Post pressure		5.20 psi	
Nominal init flow		15.2 mL/min	
Average velocity		130 cm/sec	
Inlet		Front	
Outlet (detector)		Front Detector	
Outlet pressure		ambient	

Inlet Parameters (HP-5)

<i>Front Inlet</i>	<i>(Split/Splitless)</i>	<i>Back Inlet</i>	<i>(Split/Splitless)</i>
Mode	Splitless	Mode	Splitless
Initial Temp	275 °C (on)	Initial Temp	275 °C (on)
Pressure	5.20 psi (on)	Pressure	5.20 psi (on)
Pulse time	0.60 min	Pulse time	0.60 min
Purge flow	30.3 mL/min	Purge flow	30.3 mL/min
Purge time	2.00 min	Purge time	2.00 min
Total Flow	47.9 mL/min	Total Flow	47.9 mL/min
Gas Saver	off	Gas Saver	off
Gas type	Helium	Gas type	Helium

Column Parameters

RTX-50 COLUMN (Column 3)

Capillary column			
Model number		Restek 1013174	
100% Methyl Phenyl PolySiloxane			
Max Temp		300 °C	
Nominal length		15.0m	
Nominal diameter		250.00 um	
Nominal film thickness		25.00um	
Mode		Ramped flow	
Initial flow		0.7 mL/min	
Initial Time		20.00 min	
#	Rate	Final Flow	Final time

1	0.30	1.0	10.33
2		0.0 (off)	
Post flow		0.7 mL/min	
Nominal init pressure		13.21psi	
Nominal init flow		15.4 ml/min	
Average velocity		32 cm/sec	
Inlet		Front	
Outlet (detector)		Front detector	
Outlet pressure		ambient	

Oven Parameters (6890)

Initial temp: 110 °C
Initial time: 2.00 min

Maximum temp: 300 °C
Equilibration time: 1.00 min

Ramps:			
#	Rate (C/min.)	Final Temp. (°C)	Final Time (min.)
1	15.0	280	16.67
2	0.0 (off)		
Post temp.		110 °C	
Post time		1.00 min	
Run time		30.00	

Inlet Parameters (restek)

<i>Front Inlet</i>	<i>(Split/Splitless)</i>	<i>Back Inlet</i>	<i>(Split/Splitless)</i>
Mode	Split	Mode	Splitless
Initial Temp	275 °C (on)	Initial Temp	275 °C (off)
Pressure	13.20 psi (on)	Pressure	(off)
Split Ratio:	5:1	Pulse time	(off)
Split flow:	3.5 mL/min	Purge flow	(off)
Total Flow	7.2 mL/min	Total Flow	(off)
Gas Saver	(off)	Gas Saver	(off)
Gas type	Helium	Gas type	Helium

Detector Parameters

<i>Front detector (NPD)</i>		<i>Rear detector(NPD)</i>	
Temperature	325 °C mL/min(on)	Temperature	325 °C (off)
Hydrogen flow	3.0 mL/min (on)	Hydrogen flow	3.0 mL/min (off)
Air flow	60.0 mL/min (on)	Air flow	60.0 mL/min (off)
Mode	Constant	Mode	Constant column+makeup

	column+makeup flow		flow
Combined flow	30.0 mL/min	Combined flow	30.0 mL/min
Makeup flow	On	Makeup flow	On(off)
Makeup gas type	Helium	Makeup gas type	Helium(off)
Adjust offset	30.00	Adjust offset	30.00(off)
Electrometer	On	Electrometer	(off)
Bead	On	Bead	(off)
Equilibration time	0.00	Equilibration time	0.00

Signals (Front)

Signal 1		Signal 2	
Data rate	20 Hz	Data rate	20 Hz
Type	Front detector	Type	Rear detector
Save Data	Partial	Save Data	(off)
Start Time	1.0 min	Start Time	(off)
Stop	30 min		(off)

GC Injector (7683 Series Injector)

Injector Parameters (Front)

Sample Washes	1
Sample Pumps	4
Injection Volume	1.0 microliters
Syringe Size	10.0 microliters
PreInj Solvent A Washes	0
PreInj Solvent B Washes	0
PostInj Solvent A Washes	5
PostInj Solvent B Washes	5
Viscosity Delay	0 seconds
Plunger Speed	Fast
PreInjection Dwell	0 minutes
PostInjection Dwell	0 minutes

- After reviewing instrument parameters select a "GC Real Time Plot" for viewing signal window 1 or 2 while sequence is running.

7. Make sure that under “Select Reports” all UNCHECKED.
8. Save the Method.

SEQUENCE

Prepare a sequence using the following steps.

1. Make sure the desired instrument session is up and running. Each GC has an instrument session, such as GC1, GC2, etc.
2. From the top menu select **Sequence → Load Sequence → Default.S**. Select **Sequence** again, then **Edit**. The **Sample Log Table** will open.
3. In the upper left hand corner on the **Sample Log Table**, select **Data Path**. Click on **Browse** to create a new folder under the **Data** folder of the instrument to be used.
4. In the upper right hand corner on the **Sample Log Table**, by clicking on **Browse** select the instrument **Method** to be used for analysis.
5. Type in all information under each column (i.e. Type, Vial, Sample, Method/Keyword, Datafile, Comments/Keywordstring).
6. If the Sample is a calibrator, then under **Comments/Keywordstring** type-in the MIX or Filter it is for the batch; i.e. for all levels of CAL 1 type-in MIX [A], for levels of CAL 2 type-in MIX [B] etc. *This step will be helpful during processing the data.*
7. Repeat steps 5 and 6 until all calibrators, controls, and case samples have been entered.
8. The **Datafile** name should be read GCXMMDDx001 ; where “X” is the instrument’s number, “MM” is the month, “DD” is the day, and “x” is the prefix of the sequence, such as “a”, “b” etc.
9. Once all the information is typed, select **OK** to close the **Sample Log Table**. Save the sequence by selecting **Sequence** followed by **Save Sequence As**. . . . Name the sequence as follows: GCXMMDDYY ; where “YY” defines the year.
10. Select **Sequence** then **Simulate Sequence**. In the window that opens, select **Full Method**, **Inject Anyway**, and **Overwrite Existing DataFiles**. For **Sequence Comment** enter the instrument # and detector used, the date of injection, the analyst initials, and the type of batch being injected. Select **Run Sequence**, verify all keywords by clicking “OK”.
11. Print sequence by selecting **Sequence** followed by **Print**. . .then select **Brief**. Add a “Chain of Custody” and a “Vials Loaded” label to the hard copy of the sequence.
12. Empty solvent wash bottles on the instrument and replace it with fresh methanol before starting the sequence.
13. Use the printed sequence list to load the vials in the correct position on the autosampler tray. Edit the sequence as necessary to correct any errors; save the sequence. Reprint sequence if necessary.
14. Document the “Chain of Custody” and the “Vials Loaded” labels.

15. Document the "Instrument Status Logsheet" that hangs on the instrument's oven door.
16. Start sequence by selecting **Sequence → Run Sequence**.
17. When the batch is complete, remove all vials using the sequence list. Once again document the "Vials Loaded" label.

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

Setting-Up a Subsequence

On the Sample Log Table:

1. Select "**Keyword**" for "Type"
2. Select "**DataPath**" for "Method/Keyword"
3. Under "Comment/KeywordString" type in the *new data path* for your subsequence ie: **C:\MSDCHEM\2\DATA\GCXMMDDYYx** where "X" 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; ie: GCXMMDDx001; ***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\2\DATA\GCXMMDDYYx** 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\2\DATA\GCXMMDDYYx**? → Click **Yes**.
9. A 3rd dialog box will pop-up: Sequence Verification Done! View it? → Click **Yes** or **No**.
10. Save and Print Sequence.
11. Start sequence.

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 5-11 in the "**Setting-Up a Subsequence**" Section (see previous section).

DATA ANALYSIS

Retrieving Method from Data Acquiring Instrument

1. Save the method to the batch from the acquiring instrument Chemstation.
2. Save method to batch by clicking ECM on the tool bar, then select **Save Method To ECM**. Locate the batch, select ok to save a copy of the method into the batch to be processed.
3. A copy of the method has now been saved to ECM and will accompany the batch when retrieved from ECM on any Data Analysis Processing Station.
4. Attach and document "ECM label".

PROCESSING USING ENHANCED DATA ANALYSIS

On any processing station:

1. Click on the Processing Data Analysis icon on the desktop and Log-in by using: analyst's ocme login for the username and their password.
2. From the tool bar menu select **ECM → Retrieve Entire Sequence From ECM**. Browse to locate and select the batch. Each datafile will be retrieved along with the method to be used for processing.
3. Once the retrieval is complete the batch folder is located in the C:\msdchem\ECM\retrieve folder.
4. Select the method under the batch by right clicking on it and **Load** the method.
5. Select Calibrate → Clear; clear all responses and all calibrator levels.
6. Select **Calibrate** from the tool bar, then **Update, Global Update, Set Curve Fit Type, Linear Regression Force (0, 0)**, select **OK**. If a different type of curve fit is used in place of Linear regression force (0,0), this must be annotated on the front page of the master copy of calibrators and controls that is appended to each case chromatogram.
7. Set up processing Filters (Filter A = Cal 1, Filter B = Cal 2, Filter C = Cal 3, Filter D = Cal 4, Filter E = Cal 5 etc).

- a. Select **Calibrate** again, **Edit Compounds**; select **Name**, type in compound name in the space provided, click **Find Compound**.
 - b. Change **Compound Type** from "T" to the required filter letter for that compound. *All compounds for pools or Cals 1-2 are already assigned in all master methods (i.e. the compound type for meperidine is always A, doxylamine is always B etc.) and should not have to be assigned when processing each batch.*
 - c. Click **OK** to save all changes to the compounds that have been made.
8. In order to ensure that only compounds that are found are printed in the report select **Quantitate** in the menu bar, then **Report Options**. In the lower right hand box labeled "All Reports" make sure that the box labeled "Omit Target Compounds that are Missed" is checked.
9. Create the filters needed to process the batch:
 - a. Select NYC_OCME in the menu bar, then select QDB Compound Type Filter.
 - b. Select Create and type-in as many filters as need for the batch one at a time (i.e. A, B, C, D etc). Filters are case sensitive.
 - c. Once all filters are created a "qdb filter" window automatically pop-up with a list of all the compounds in the filters needed to process the batch.
10. Load the first calibrator at the highest level (i.e. 3.0 mg/L) by selecting **File** → **Load Data File**
11. Load the filter needed for the data file:
 - a. Select **NYC_OCME** in the menu bar, then select **Compound Type Filter**
 - b. Type the # for that filter, where 1 is the first assigned filter.
12. In the space provided type in **QT 1**, then click on **Execute**; this processes the data file.
13. Select the **View** from the top menu, select **Easy ID** (this function allows for Retention times to be assigned for the entire method and batch). *Easy ID should only be used for your first calibrator level.*
14. To select a compound from the list double click, use the right click to integrate a peak for the compound, use the left click to control zooming on the chromatogram.
15. Once all of the retention times have been assigned, click on **Exit** and click on **Yes** to "update retention times to the compound list".
16. Type in **QT 1**, then click on **Execute**.
17. Again select the **View** from the top menu, select **Q-Edit Quant Result** (this function to view the response and retention times for each compound.) *Cal 3.0 mg/L will not have an "x" next to each compound name because it is the first calibrator to be processed.* Click **Exit** when all compounds have been reviewed.
18. Assign the calibrator level:
 - a. Select **Calibrate**, then **Update**, select **Update 1 Level**.

- b. Select **Add Level** and type in the concentration of the calibrator (i.e. 3.0, 1.0 etc.).
 - c. Type-in the internal standard concentration (1.0).
 - d. On the right hand side of the window type-in the Level ID i.e. 4A, 3A, and 2A, 1A; where the # is the calibrator level and the letter is the filter.
 - e. Click on **Do Update** then select **OK**.
19. Load the next data file, Cal 1.0 mg/L. Type **QT 1** then click on **Execute**.
20. Select the **View**, select **Q-Edit Quant Result**. *Easy ID is not needed for this step, as retention times have already been set for this calibrator.* Double check each of the compound's retention time and that each compound has a response. All auto- integrated compounds should have an "x" next to their name. Click **Exit** once when finished.
21. Assign the calibrator level.
22. Repeat steps 15-21 for the remaining levels (0.2 and 0.05 mg/L) for the first calibrator.
23. Requant the first calibrator:
- a. Go to **Tools, DOLIST**.
 - b. Remove any options that are on the right hand side of the box.
 - c. On the left hand menu select "**Summary Quant No Report**" which is **QT 2**, with arrows move this command to the empty space on the right then click **OK**.
 - d. Select the calibrators just processed and move them to the empty space on the right then click **Process**. Each file will be "requanted".
 - e. Review all files through **Q-Edit Quant Result** to verify quant results.
24. Process the corresponding QC's for the calibrator that was just reviewed, with the same filter on:
- a. Go to **Tools, DOLIST**.
 - b. Remove any options that are on the right hand side of the box.
 - c. On the left hand menu select "**Quant No Report**" which is **QT 1**, with arrows move this command to the empty space on the right then click **OK**.
 - d. Select the QC's to be processed and move them to the empty space on the right then click **Process**. Each file will be processed.
25. Review each QC by selecting **View**, select **Q-Edit Quant Result**.
26. With the filter still on, print the Cal's and QC's for that filter:
- a. Go to **Tools, DOLIST**.
 - b. Remove any options that are on the right hand side of the box.
 - c. On the left hand menu select "**Summary Quant w/o Calculations**" which is **QT 0, 1, "s"**, with arrows move this command to the empty space on the right then click **OK**.
 - d. Only select all Cal levels and QC's just reviewed and quanted and move them to the empty space on the right then click **Process**. Each file will print.

27. Load the 3.0mg/L of the next calibrator to be processed and follow steps 10-26.
28. Once all calibrators and QC's are printed, save the method to ECM, then **set the filter # to 0, which gives a list of all the compounds for the entire method**, and process the all the blank injections and cases:
- Go to **Tools, DOLIST**.
 - Remove any options that are on the right hand side of the box.
 - On the left hand menu select "**Quant No Report**" which is **QT 1**, with arrows move this command to the empty space on the right then click **OK**.
 - Select all blanks and cases to be processed and move them to the empty space on the right then click **Process**. Each file will be processed.
29. Review each blank and case through **Q-Edit Quant Result**:
- Deselect any compounds that are not needed in each blank or case by double clicking on it and clicking on **QDel**.
 - Check the responses and retention times of the needed compounds.
 - If a compound is needed but not integrated, double click on the compound and integrate.
 - Click **Exit** once when finished, click "yes" to the save changes dialog box.
30. If a compound is too low to be integrated or no peak is present include an overlay:
- Go to **Tools, Overlay Chromatogram**.
 - Select the calibrator or standard to overlay on the case; multiple overlays can also be set.
 - Select **Process**.
 - Go to **File, Print** then select **Chromatogram**.
31. Once all blanks and cases are processed, print as directed in step # 26a-c.
32. Print an R^2 report by:
- Go to **Calibrate, List** then select **Calibration Report** and **OK**.
 - Compounds that were not used in the batch can be deleted by highlighting them and simply pressing delete on the keyboard. *Caution should be used, do not remove compounds needed.*
 - Right click on the list and select **Print**, choose the correct printer.
33. Once everything is printed, save the all the data files from the batch back to ECM:
- Select **ECM** from the top tool bar, select **Save Multiple Data Files to ECM**.
 - Select all data files on the left and move them to the right empty space, the click **Process**.
 - Allow each data file to be successfully copied to ECM before exiting Data Analysis.

34. After uploading the files to ECM, delete the sequence from the C: Drive of the processing station:

- a. Right click on the **Start** on the lower left hand corner and select **Explore**
- b. Open the **MSDCHEM** folder on the **C: Drive**, then select **1 → ECM → Retrieve**
- c. Locate the batch processed, right click on it and delete it.
- d. Document "ECM label" on sequence list.

RE-INJECTION CRITERIA

Occasionally, samples analyzed may need to be reinjected for a variety of reasons. The criteria for reinjection, other than poor chromatography or requests made by the appropriate supervisor, are listed below.

1. Individual blood samples may be re-injected once if internal standard (IS) area counts show poor recovery.
2. Analytes in a calibrator or control are not present in the chromatogram.
3. Additional peaks present in the chromatogram of a calibrator or control. See TROUBLESHOOTING OF AGILENT GAS CHROMATOGRAPHS section of the SOP manual.
4. QC failure: reinject the blank, the calibrator of interest and the associated QC.
5. Examine peak shape for signs of overload. If peaks are still overloaded after reinjection, re-extract samples with overloaded peaks using appropriate dilutions. If a calibrator peak is still overloaded after re-injection, the batch must be re-extracted.

CARRYOVER

Two types of carryover are possible with the methods employed by GC. One is the "carryover" of late-eluting peaks from one injection into the next or even next two or three injections. This is usually apparent from the shape of the peak (broad).

The other possibility is "carryover" due to contamination from an excessive amount of drug in the previous sample, either through the syringe or by drug being retained in the injection port or column. This is controlled by the fact that all positive screens are repeated with a new aliquot, to quantitate. Also, the original screen results are compared with the quant results. Any discrepancy is resolved, usually by repeating the extraction. Any samples with large concentration of drug are rescheduled with dilutions, to reduce the amount to within the linear range of the method. If it appears that carryover of this type has occurred, the reviewer will reschedule the case after the overload to verify that there was no contamination.

Frequent change of the autosampler methanol wash bottles helps to control this type of potential carryover.

ACCEPTANCE CRITERIA

1. External or internal controls must be analyzed in accordance with the standard laboratory procedures to determine the target and acceptable range before being incorporated into routine analysis of the specimens.
2. None of the target analytes should be detected in the negative control (blank).
3. Retention times of target analytes should match those of the calibrator or controls within $\pm 2\%$.
4. The positive controls, unless otherwise specified, should be within $\pm 20\%$, for blood batches, or $\pm 30\%$, for tissue batches, of the established target for all analytes detected in the samples. In other words, if amitriptyline, nortriptyline and methadone are present in the samples, the respective components of the controls must meet the acceptance criteria, but it is not necessary for the other compounds in that same control to meet the acceptance criteria. If the QC fails for quantitation, but is correct qualitatively, a batch can be accepted for qualitative results only. Acceptance of controls outside of $\pm 20\%$ blood or $\pm 30\%$ for tissues, of the established target require authorization of the supervisor and documentation.

DATA REVIEW

(Second level review)

1. Check chromatography of all injections. Examine the peak shape and note if any peaks show non-Gaussian shape. Identify unresolved peaks and peaks with shoulders on either side. Address any noted problems before proceeding with data review. Consult a supervisor about any unusual events.
2. Check that all significant peaks in the chromatogram are integrated. If the printout allows, check if the baseline used to integrate is appropriate. The analyst should consult with a supervisor about any unusual events, such as the presence of overload peaks
3. Check that all components of each calibrator are present and that each peak is properly assigned. Correct any errors. If any calibration compounds do not extract, or if unexpected multiple peaks are detected for a calibration compound, the analyst must consult with a supervisor.
4. Check the blank for significant peaks that might co-elute with a compound of interest. There is some room for judgment in this step, but the analyst should consult with a supervisor if there are ANY questions. A blank may be scheduled for mass spectrometry to discover the identity of peaks in a blank, but this step must have no bearing on the decision whether a blank is acceptable or not. If any significant peaks are present in a blank for the batch, the run must be rejected.
5. Review QC samples, determine if the controls meet all acceptance criteria and verify the concentration of the components.

6. A copy of the batch calibrators and controls are forwarded to the QC officer, who will enter the results of the QC sample in the QC database. If the batch fails, annotate sample chromatograms, listing the reason for failure.
7. Annotate the following information on the first calibrator's chromatogram or sequence list:
 - a. Calibrators and blank reviewed and accepted (or not accepted). Any QC or calibrator failures are annotated on the proper chromatograms.
 - b. If the files did not transfer, do so, and notify supervisor.
 - c. Note any deviations from the SOP or any comments in a concise but detailed fashion.
 - d. Initial and date.
 - e. Regression coefficient (r^2) must be greater than or equal to 0.98.
8. Make sure that each control has the appropriate target concentration range label.

Note: *By initialing and dating this document, the analyst certifies that a complete and accurate review was done, to the best of the ability of the analyst.*

9. A supervisor or qualified staff reviews calibrators and controls, dates and initials the results of the review before photocopying.
8. When possible, all data should be photocopied using double sided mode. A master copy of the sequence list, calibration, control and any other data is prepared. Arrange this master copy as follows (from top to bottom): Calibrators, all QC samples and blanks, r^2 report, sequence list and the lot sheet form. It is the responsibility of the analyst to verify that the master copy is legible and is an accurate copy of the originals, with no information cut off at the margins. Photocopies are made from this master, one for each case in the batch. Attach a copy of the calibrator and control package to each original case chromatograph.

DATA REVIEW

(Third level review)

Quantitative Results

1. The reviewer should review the calibration, control, and batch information received after the photocopying of the batch. Use the same criteria as listed in batch review, with the additional criteria that the r^2 of the four-point calibration curve of each component present in any case in the batch must be equal to or greater than 0.98. Notify a supervisor about any errors and make sure they are corrected before proceeding.
2. Retrieve the case file for each sample in the batch and associate it with the chromatogram(s).
3. Review the case's chromatogram. Determine if the results are consistent with all the other applicable case data to date. For quantitation, it is important to compare the first chromatogram (or the SCAN) results with the final quantitative results. The ratio of peak to

internal standard for both chromatograms should be examined. If the results are consistent, report the results on the Result Summary Sheet. Date and initial both the chromatogram and the Result Summary Sheet. If the results are not consistent, the inconsistency must be resolved. Consult a supervisor about rescheduling the sample, or other appropriate corrective action.

4. If the chromatogram shows overloaded peaks, or poor recovery of the internal standard, then repeating the sample may be necessary. If the problem is an overload, re-schedule with appropriate dilutions. In cases of poor recovery, the sample, its control, blank, and a calibrator may be re-injected once. If the criteria as listed in batch review are still not met, the sample is re-scheduled. If any cases have an unknown or unidentified peak not observed in previous scan results, the case vials must be transferred to GC/MS for identification. All chromatograms must be attached to the case file. Consult with the supervisor if there is any question.

Note: Do not discard any sample paperwork.

5. Cases where an overdose is suspected are scheduled for further testing. Liver and gastric contents are the specimens of choice. Consult with the supervisor regarding tissue analysis.

REPORTING

1. All control acceptance criteria must be met.
2. Only specimens that have been run along with acceptable controls may be reported. Report concentrations equal to or higher than 50% of the lowest acceptable control or the lowest acceptable calibrator. Concentrations equal to or greater than 25% of the lowest control or lowest calibrator but lower than 50% of the lowest acceptable control or the lowest acceptable calibrator are reported as "less than X" where "X" equals the concentration of the lowest acceptable calibrator or control. Concentrations lower than 25% of the lowest acceptable control or calibrator are reported as "not detected".
3. All other positive findings are reported to the hundredth of mg/L, (e.g., "metoclopramide 0.82 mg/L").
4. Drugs detected at a concentration above the upper limit of quantitation must be reported as "greater than...", unless the concentration was determined by repeat analysis using appropriate dilutions. Diluting until the sample is within the linear range of the analysis is the most desirable result, but if the sample is insufficient, or other considerations preclude dilution, report as above.
5. Drug facilitated sexual assault cases and driving under the influence cases are reported in ng/mL
- 6.

REFERENCES

SPEware Corp. Cerex Applications Manual.

System 48 Processor. Users Guide.

Turbovap. Users Guide.

Uncontrolled Copy