FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
NEW YORK CITY

BASIC DRUGS

QUANTITATION by NPD GAS CHROMATOGRAPHY

Uncontrolled Copy

PRINCIPLE

Basic drugs encompass the largest group of compounds analyzed at the OCME FTL. This procedure is designed to extract basic drugs from biological specimens for analysis by gas chromatography (GC) using a nitrogen-phosphorus detector (NPD). The procedure is used for quantitative purposes.

Basic drugs are extracted from biological fluids or tissue homogenates by adjusting the matrix pH to 9.8 and extracting the drugs with n-butyl chloride. Drugs are back-extracted from n-butyl chloride into an acid; the aqueous solution is made basic and extracted with a small volume of organic solvent which is analyzed by GC NPD.

A four-point calibration curve is used for routine all analyses (0.05 mg/L, 0.20 mg/L, 1.0 mg/L and 2.0 mg/L) along with a negative control and three positive controls (0.025 mg/L, 0.5 mg/L and 1.0 mg/L). The low control (0.025 mg/L) challenges the assay near the lower limit of quantitation and the high control (1.0 mg/L) challenges the assay near the upper limit of quantitation.

SAFETY

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

REAGENTS AND MATERIALS

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

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

   Slowly add 212 g of Na_2CO_3 to 2500 mL of distilled water in a 4000 mL Erlenmeyer flask. Once this is completely dissolved, slowly add 168 g of $NaHCO_3$. Dilute to the mark with distilled water and mix on a stirring plate for one hour. Using a pH meter, verify that the pH is in fact 9.8. If the pH needs adjustment, use acid or base as needed to adjust to 9.8, and document on the solution preparation sheet that the pH was checked. Date and initial the preparation sheet.
3. **n-Butyl chloride (1-Chlorobutane)**

4. **0.5N HCl**

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

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

5. **Sodium carbonate**

Slowly add 530 g of Na_2CO_3 to 3000 mL of distilled water in a 4000 mL Erlenmeyer flask. Dilute to the mark with distilled water and mix on a stirring plate for one hour.

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

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

7. **Calibrators and controls**

Calibrators and in-house controls are prepared according to the section "Preparation of GC Calibrators and Controls". Internal controls may be used to fulfill the requirement of the 1.0 mg/L, 0.5 mg/L and 0.025mg/L controls for each batch. Additional controls, as dictated by respective assays, may be included.

8. **Methapyrilene Internal Standard, 50 mg/L.**

See SOP section ""Preparation of GC Calibrators and Controls" for preparation.

9. **50% Sodium Hydroxide solution (Fisher Scientific or equivalent)**

(for propoxyphene/norpropoxyphene analysis)

SPECIMEN PREPARATION

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

Bile	2 mL of a 1:5 dilution.
Blood	2 mL of undiluted specimen.
Brain	2 mL of a 1:3 homogenate with DI water.
Gastric Contents	2 mL of a 1:10 dilution with DI water.
Liver	2 mL of a 1:5 homogenate with DI water.
Urine	2 mL of undiluted specimen.

DILUTION OF SPECIMENS

Specimens are diluted as follows:

Bile 1:5	1.0 mL of undiluted bile *q.s.* to 5 mL with deionized water.
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.

Uncontrolled Copy

Gastric Contents 1:10	2.0 mL of liquid *q.s.* 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: *The entire submitted amount of gastric contents must be weighed prior to homogenizing and sampling; total weight must be documented on reports.*

Ensure Solid Samples are homogenized first.

EXTRACTION PROCEDURE

Note: If extracting any of the following, methadone metabolite, propoxyphene/norpropoxyphene, quinine/quinidine, or zopiclone refer to the section after the extraction procedure of this SOP for the necessary modifications.

1. Print a worklist of cases to be analyzed. Review with supervisor for any updates of priority (RUSH) cases or other special instructions. Review the drugs present in the cases selected. Include calibrators for each group that has a drug from any case in the batch. If the cases contain any drugs that are not in pools 1, 2 calibrators and their corresponding controls, then additional calibrators and controls must be prepared and analyzed with the batch. The batch size limit may be exceeded by authority of the section supervisor at need. Every drug present in a case in a batch must be included in one of the calibrator pools and in the corresponding controls.
2. Find and collect all samples on work list, and place in an empty specimen rack.
3. Obtain enough 16 x 150 mm screw-cap culture tubes (two per aliquot) and Teflon-lined caps to extract negative control, calibrators, controls and all requested case work. Label the tubes appropriately. Tubes should bear the entire toxicology number (e.g., 99-1234, **not** 1234), the specimen type and any appropriate dilution. If a sample matrix other than the one used for the calibrators is analyzed in a batch, include a blank and controls using that matrix.
4. Obtain the required calibrators and controls for pools 1 and/or 2 from the refrigerator and allow them to equilibrate to room temperature. After making note of the lot numbers on the lot form sheet, place these samples in random positions within the casework tubes. Prepare any required additional calibrators.
5. Pipet 2.0 mL of a matching, negative matrix into each of the blank and calibrator tubes (the negative matrix used for gastric, urine and vitreous humor samples is deionized water). Cover the tubes with a cap to avoid possible contamination from other specimens. Fortify the tubes with various solutions as indicated.
6. For Group 1 & Group 2, fortify each tube with the needed stock solutions as indicated below:

Final Concentration (mg/L)	Stock Solution Concentration (mg/L)	Spiking Volume (µL)
0.025	10	5
0.05	10	10

Uncontrolled Copy

0.2	10	40
0.5	100	10
1.0	100	20
2.0	100	40

7. For additional drugs not found in groups 1 or 2, fortify each tube with the needed amounts listed below

Final Concentration (mg/L)	Stock Solution Concentration (mg/L)	Spiking Volume (µL)
0.025	10	5
0.05	10	10
0.2	10	40
0.5	100	10
1.0	100	20
2.0	100	40

INTERNAL STANDARD

In rare instances, methapyrilene and asenapine are unsuitable as the internal standard. In such cases, a different analyte may be used as an internal standard (i.e. promazine or loxapine). These exceptions must be noted on the sequence list.

***Note**: Additional calibrators and controls may need to be prepared. A four-point calibration for each drug of the additional calibrator(s) must be made, along with appropriate controls, containing every drug needing quantitation and not present in the available pools of calibrators and controls already prepared. Drugs may be added to the existing calibrators and controls if they are well resolved from the other drugs in that calibrator and control. In case of doubt, discuss with a supervisor or manager. If the retention time of a drug is in question, a methanolic solution of the drug should be injected on the GC planned for the batch to determine the retention time of the drug in question. See "Preparation of GC Calibrators and Controls" for details.*

8. After all calibrators and controls have been prepared, aliquot the case specimens. Mix each specimen container by gentle inversion and pipet 2.0 mL of each specimen into the appropriately labeled tube. Make certain that the toxicology number on the specimen bottle exactly matches the number on the tube. Cap each tube after pipetting to prevent contamination from other cases. If a blood specimen is being pipetted and a clot is encountered, homogenize the specimen using the Polytron or a glass manual homogenizer. The analyst documents on the blood bottle that it was homogenized and annotates on sequence list and the Case Analysis Form in the comments section. Continue in this fashion until all cases have been pipetted.
9. Enter the date extracted in the DataEase database for each case picked up in the batch.

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

Uncontrolled Copy

10. Fortify, using a syringe, 40 µL of 50 mg/L methapyrilene internal standard to tubes to achieve a final concentration of 1.0 mg/L.
11. Pipet 1.0 mL of the pH 9.8 buffer into each tube. Vortex each tube to thoroughly mix. Add 4.0 mL of n-butyl chloride into each tube and cap all tubes. Briefly invert each tube to check for leaks. If a leak is found, transfer the tube contents to another appropriately labeled tube. Place all tubes on the mechanical shaker for 10 minutes using a low setting. Remove the tubes from the shaker and centrifuge for 10 minutes at ≈ 3000 rpm. If an emulsion is noticed, break up the emulsion by shaking or using a sterilized cotton tipped applicator, and re-centrifuge. If the emulsion still persists, see the supervisor for alternate methods.
12. Transfer the upper, organic layer of each tube to a new appropriately labeled tube which contains 2.0 mL of 0.5N HCl. Make sure that the number on the two tubes match. Handle only one set of tubes at the same time. Cap and briefly invert each tube to check for leaks. Place on the mechanical shaker for 10 minutes on a low setting. Remove the tubes from the shaker and centrifuge for 10 minutes at ≈ 3000 rpm.
13. Upon removal from the centrifuge, uncap each tube and aspirate the upper, organic layer to waste using a suction/aspiration apparatus. Pipet 1.0 mL of sodium carbonate solution into the lower acid layer. Pipet 1.0 mL of the pH 9.8 buffer into each tube and briefly vortex briefly.

Note: *Addition of the sodium carbonate will cause release of CO_2 during the neutralization reaction which will result in foaming.*

14. Add 200 µL of the toluene/heptane/isoamyl alcohol solution (THI) into each tube. Cap all tubes and place on the mechanical shaker for 10 minutes on a **high** setting. Remove the tubes from the shaker and centrifuge for 10 minutes at ≈ 3000 rpm. Using a Pasteur pipet, transfer the upper THI layer to a glass insert in an appropriately labeled vial.

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

15. 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. Physically check that the crimped seal is tight by attempting to rotate the seal. Crimp until tight, using a new seal if necessary. Do not wait until all transfers have been made to seal the vials. Transfer the sealed vials to the GC Instrumentation Laboratory and continue with analysis by gas chromatography.

ANALYSIS WHERE METHADONE METABOLITE IS PRESENT

Note: *Methapyrilene co-elutes with the primary methadone metabolite (EDDP). Asenapine, loxapine or promazine may be used as an alternate internal standard providing that those analytes were not detected in the qualitative screen. These internal standard solutions will be prepared at a concentration of 100 mg/L and the analyst will need to fortify 20 µL.*

1. Add one of the alternate internal standards to achieve a final concentration of 1.0 mg/L in all instances where internal standard is added to extraction tubes.

Note*: Do not add methapyrilene to any test tube!*

Uncontrolled Copy

2. Process, review, and report as all other quant batches. *Remember to change the internal standard from methapyrilene to promazine or other compound used as an alternative internal standard in the processing method.*

PROPOXYPHENE AND NORPROPOXYPHENE ANALYSIS

At step 13 of the extraction procedure, after adding pH 9.8 buffer, add enough 50% sodium hydroxide drop-wise to bring the pH of each test tube to 11. Use the appropriate pH paper or indicator strips to check the pH of each test tube contents, Continue with step 14 of the extraction procedure.

QUININE/QUINIDINE ANALYSIS (n-BUTYL CHLORIDE EXTRACTION)

At step 14 of the extraction procedure, **instead** of THI, add 200 µL of n-butyl chloride to each test tube, shake on high, and then centrifuge as previously directed. Transfer the n-butyl chloride into an appropriately labeled vial with glass insert. Completely dry the sample on a drying block that uses N_2. Reconstitute by adding 50 µL of methanol to the glass insert. The sample is ready to be analyzed by GC/NPD.

ZOPICLONE ANALYSIS

Zopiclone is a short-acting non-benzodiazepine hypnotic drug prescribed for insomnia. In this procedure, this drug is injected at 180 ^{0}C due to thermal breakdown in the inlet area.

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 µm 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 µm 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 0.25µm film thickness). This column is used as needed to analyze compounds that co-elute; consult with a supervisor.

INSTRUMENT SETUP

6890 Series II Gas Chromatogram

Uncontrolled Copy

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 the data acquiring computer (Chemstation) verify that all 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

Uncontrolled Copy

Front Inlet	*(Split/Splitless)*	*Back Inlet*	*(Split/Splitless)*
Mode	Splitless	Mode	Splitless
Initial Temp	275 ^{0}C (on)	Initial Temp	275 ^{0}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

HP-17 COLUMN (Column 1)

Capillary column			
Model number			19095L-121
HP-50+ 50% Phenyl Methyl Siloxane			
Max Temp			310 ^{0}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 ^{0}C
Initial time: 2.00 min
Maximum temp: 280 ^{0}C
Equilibration time: 1.00 min

Ramps:			
#	**Rate (C/min.)**	**Final Temp. (^{0}C)**	**Final Time (min.)**
1	15.0	280	18.00
2	0.0 (off)		

Uncontrolled Copy

Post temp.	120 ^{0}C	
Post time	1.00 min	
Run time	30.67	

Detector Parameters

Front detector (NPD)		*Rear detector(NPD)*	
Temperature	325 ^{0}C mL/min(on)	Temperature	325 ^{0}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, Rear, 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 ^{0}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

Uncontrolled Copy

#	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)

Front Inlet	*(Split/Splitless)*	*Back Inlet*	*(Split/Splitless)*
Mode	Splitless	Mode	Splitless
Initial Temp	275 ^{0}C (on)	Initial Temp	275 ^{0}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 ^{0}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	

Uncontrolled Copy

Outlet (detector)	Front detector	
Outlet pressure	ambient	

Oven Parameters (6890)

Initial temp: 110 ^{0}C
Initial time: 2.00 min
Maximum temp: 300 ^{0}C
Equilibration time: 1.00 min

Ramps:			
#	**Rate (C/min.)**	**Final Temp. (^{0}C)**	**Final Time (min.)**
1	15.0	280	16.67
2	0.0 (off)		
Post temp.	110 ^{0}C		
Post time	1.00 min		
Run time	30.00		

Inlet Parameters (restek)

Front Inlet	*(Split/Splitless)*	*Back Inlet*	*(Split/Splitless)*
Mode	Split	Mode	Splitless
Initial Temp	275 ^{0}C (on)	Initial Temp	275 ^{0}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

Front detector (NPD)		*Rear detector(NPD)*	
Temperature	325 ^{0}C mL/min(on)	Temperature	325 ^{0}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 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(off)
Makeup gas type	Helium	Makeup gas type	Helium(off)
Adjust offset	30.00	Adjust offset	30.00(off)
Electrometer	On	Electrometer	(off)

Uncontrolled Copy

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	3.0 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

6. After reviewing instrument parameters select a “GC Real Time Plot” for viewing signal window 1 or 2 before running the batch; Signal 1 and/or Signal 2Attn 8, Offset 10%, Time 30 min
7. Save the Method.

SEQUENCE

Prepare a sequence using the following steps.

Uncontrolled Copy

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. (GCx030413A..)
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, Multiplier).
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 GCXMMDD**x**00**1** ; 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" of "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", "Vials Loaded" label and "save method to ECM" label to the hard copy of the sequence.
12. Empty solvent wash bottles on the instrument and refill 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.*

Uncontrolled Copy

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\GCXMMDDYY*x*** 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. Create a new folder for subsequence.
5. The suffix of the data files must be different from that of the original; ie: GCXMMDD**x**00**1**; ***the subsequence data files must start with 1 again***.
6. After typing in the entire sequence, save sequence accordingly and method your using.
7. Go to Sequence → Simulate Sequence → Run Sequence.
8. A dialog box will pop-up: DataPath **C:\MSDCHEM\2\DATA\GCXMMDDYY*x*** does not exist. Edit Sample Log Table? → Click **No** if the sequence was set up correctly.
9. A second dialog box will pop-up: Create **C:\MSDCHEM\2\DATA\GCXMMDDYY*x***? → Click **Yes**.
10. A third dialog box will pop-up: Sequence Verification Done! View it? → Click **Yes** or **No**.
11. Save and print sequence.
12. Log into LIMS with your ID and password.
13. Create a LIMS sequence in the instrument usage log.
14. Start sequence.

DATA ANALYSIS

Retrieving Method from Data Acquiring Computer (Raw Data)

1. From the instruments' computer save the method to the batch.
   a. 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.
2. 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.
3. Attach and document "ECM label" on the sequence list.

Uncontrolled Copy

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 Manager. 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.

ANALYSIS - FIRST LEVEL REVIEW

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 data file 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. Make sure retention window setting is set to ± 2%.
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).
   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. Create the filters needed to process the batch:
   a. Select NYC_OCME in the menu bar, then select QDB Compound Type Filter.

Uncontrolled Copy

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.

9. Load the first calibrator at the highest level (i.e. 3.0 mg/L) by selecting **File → Load Data File**
10. 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.
11. In the space provided type in **QT 1**, then click on **Execute**; this processes the data file.
12. 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.*
13. 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.
14. Once all of the retention times have been assigned, click on **Exit** and click on **Yes** to "update retention times to the compound list".
15. Type in **QT 1**, then click on **Execute**.
16. Again select the **View** from the top menu, select **QEdit 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.
17. 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**.
18. Load the next data file, Cal 1.0 mg/L. Type **QT 1** then click on **Execute**.
19. Select the **View**, select **QEdit 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 there name. Click **Exit** once when finished.
20. Assign the calibrator level.
21. Repeat steps 15-21 for the remaining levels (0.2 and 0.05mg/L) for the first calibrator.
22. Requant the first calibrator:

    a. Go to **Tools**, **DOLIST.**

    b. Remove any options that are on the right hand side of the box.

Uncontrolled Copy

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 **QEdit Quant Result** to verify quant results.

23. 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.

24. Review each QC by selecting **View**, select **QEdit Quant Result**.

25. 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.

26. Load the 3.0mg/L of the next calibrator to be processed and follow steps 10-26.

27. 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, then*** process all blanks and cases:

    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 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.

28. Review each blank and case through **QEdit Quant Result**:

    a. Deselect any compounds that are not needed in each blank or case by double clicking on it and clicking on **QDel**.

    b. Check the responses and retention times of the needed compounds.

    c. If a compound is needed but not integrated, double click on the compound and integrate.

    d. Click **Exit** once when finished, and click "YES" to save the changes dialog box.

29. If a compound is too low to be integrated or no peak is present include an overlay:

Uncontrolled Copy

a. Go to **Tools**, **Overlay Chromatogram.**

b. Select the calibrator or standard to overlay on the case; multiple overlays can also be set.

c. Select **Process.**

d. Go to **File**, **Print** then select **Chromatogram.**

30. Once all blanks and cases are processed, print as directed in steps # 25 a-c.
31. Print an R^2 report by:

a. Go to **Calibrate**, **List** then select **Calibration Report** and **OK**.

b. 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.*

c. Right click on the list and select **Print**, choose the correct printer.

32. Once everything is printed, save the all the data files from the batch back to ECM:

a. Select **ECM** from the top tool bar, select **Save Multiple Data Files to ECM.**

b. Select all data files on the left and move them to the right empty space, the click **Process**.

c. Allow each data file to be successfully copied to ECM before exiting Data Analysis.

33. 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 the sequence list.

REINJECTION 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 along with their respective calibrators and controls may be reinjected 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, affected cases and the associated QC. If upon re-injection there is still a QC failure schedule the cases for re-extraction, make a note in DataEase and on the appropriate Case Analysis Form(s).

Uncontrolled Copy

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.

6. Consult with a supervisor for any unusual events or if you need assistance in evaluating the chromatography of a sample for re-injection.

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, any level 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. All 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. The positive controls, unless otherwise specified, should be within ± 20%, for blood batches, and ± 30%, for tissue batches, of the established target for all analytes detected in the samples. In other words, if amitriptyline, nortriptyline and diphenhydramine 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. After re-injection, if the QC fails for quantitation, but is correct qualitatively, a batch can be accepted for qualitative results only. Acceptance of controls outside of ± 20% blood and ± 30% for tissues, of the established target require authorization of the supervisor or manager and documentation.
3. None of the target analytes should be detected in the negative control (blank).
4. Retention times of target analytes should match those of the calibrator or controls within ± 2%. The analyst shall choose from the standard levels a level at their discretion. All internal standard areas/peak heights for that sequence shall be compared to the internal standard(s) in that level. The acceptance criteria for the internal standard recovery/response shall be ± 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.

Uncontrolled Copy

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 ± 20% of their average responses; the calculated amount of the spiked sample is ± 20% of the theoretical amount.

If any of the above conditions are not met than consult with a toxicology manager for further direction.

PREPARING DATA FOR SECOND LEVEL REVIEW

1. Ensure no carryover; if carryover is present resolve before proceeding**.**

2. If the batch and method files did not transfer, do so, and notify supervisor.

3. Annotate the following information on the first calibrator’s chromatogram or sequence list:

a. Calibrators, controls and blank review, if the batch fails, annotate sample chromatograms, listing the reason for failure; notify a supervisor and / or the QC manager.

b. Note any deviations from the SOP or any comments in a concise but detailed fashion.

c. Regression coefficient (r^2) must be greater than or equal to 0.98.

d. Make sure that each control has the appropriate target concentration range label.

e. Initial and date.

f. Each case must have a copy of the sequence list, calibration, control, internal standard recovery form and any other relevant data. Arrange this master copy as follows (from top to bottom): Calibrators, - QC samples,, internal standard recovery form. 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. Attach a copy of the calibrator and control package to each original case chromatograph, one for each case in the batch.

g. Submit data for Second Level Review.

Note: By initialing and dating this document, the analyst certifies that a complete and accurate review was done

SECOND LEVEL REVIEW

The Second Level Reviewer will review the processed data in its entirety according to the acceptance criteria. The second level reviewer will also ensure the following;

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. Consult a supervisor about any unusual events.

Uncontrolled Copy

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, ensure that the sample chromatograms are annotated, listing the reason for failure. Notify a supervisor and / or the QC manager.
7. Ensure the following information is annotated 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. Any deviations from the SOP are noted or any comments are stated in a concise but detailed fashion.
    c. Initial(s) and date
    d. Regression coefficient (r^2) of the four point calibration curve of each component present in any case in the batch must be greater than or equal to 0.98. Notify a supervisor about any errors and make sure they are corrected before proceeding.
8. Make sure that each control has the appropriate target concentration range label.
9. Review calibrators, controls, and blanks; ensure all acceptable criteria are met.
10. Initial and date the results.

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

11. Ensure the master copy of the sequence list includes, (from top to bottom): Calibrators, all QC samples and blanks, r^2 report, sequence list, internal standard recovery form 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.

11. Retrieve the case file for each sample in the batch and associate the case file with the corresponding chromatogram(s).

Uncontrolled Copy

12. Review the case's initial chromatogram, if applicable. 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.
13. 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 a case has an unknown or unidentified peak not observed in previous scan results, the case must be transferred to the GCMS section for identification of that peak. All chromatograms must be attached to the case file. Consult with the supervisor if there is any question.
14. Submit the case file for third level review.

Note: *Do not discard any sample paperwork.*

THIRD LEVEL REVIEW (FINAL REVIEW)

The third and final level review can only be performed by the Laboratory Manager. 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.

REPORTING

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

1. All control acceptance criteria must be met according to the guidelines.
2. Analytes detected at a concentration of 0.05 mg/L (lowest calibrator) or 0.025 mg/L (lowest control) are reported as "< (less than…)" the lowest calibrator or control (i.e. if the analytes concentration is < 0.025 mg/L, report as less than 0.025 mg/L).
   a. If 0.025 mg/L control passes and the analytes concentration is greater than the 0.025 mg/L control, report the numerical value.
   b. If 0.025 mg/L control fails and the lowest calibrator (0.05 mg/L) passes report the analytes concentration as less than 0.05 mg/L.
   c. If the lowest calibrator and control fail the case must be repeated.

Uncontrolled Copy

3. All reported concentrations less than 1.0 mg/L are reported to the hundredth of a mg/L, (e.g., “Amitriptyline 0.82 mg/L”). Positive findings ≥ 1.0 mg/L are reported to the tenth of a mg/L (e.g. “Amitriptyline 1.8 mg/L”)
4. Drugs detected at a concentration above the upper limit of quantitation must be repeated with appropriate dilutions. Dilutions are performed until the results are within the linear range of the calibration curve. If the sample is insufficient or other considerations preclude dilution, consult with a supervisor.
5. Drug facilitated sexual assault cases and driving under the influence cases are reported in ng/mL.

REFERENCES

1. E. H. Foerster, P. Hatchett and J. C. Garriott, “A rapid, comprehensive screening procedure for basic drugs in blood or tissues by gas chromatography”, Journal of Analytical Toxicology, Vol.2, No.2, pp 50-55, 1978.

Uncontrolled Copy

REVISION HISTORY

Ver 03.08.2013	1. Revision history implemented.
Ver 06.11.2013	1. Modified PRIMARY METHADONE METABOLITE ANALYSIS to include use of alternate internal standard
Ver 10.07.2013	1. Volumes and weights of specimen requirements are reduced along with the corresponding extraction solvent volumes.
Ver 04.20.2015	1. Minor grammatical & spelling corrections. 2. Removal of Methadone and EDDP from quantitative analysis (transferred to GC/MS analysis).
Ver. 04.26.15	1. Removal of automatic scheduling of liver or gastric specimens with apparent overdose concentratons. 2. Clarification of quantitative reporting (number of decimal places.
Ver 09.14.2015	1. Clarification of 1st, 2nd and 3rd review. 2. Change the calibration range, 3. Defined acceptance criteria.

Uncontrolled Copy