

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

**SCREENING FOR BASIC DRUGS BY GAS CHROMATOGRAPHY WITH
SIMULTANEOUS NITROGEN-PHOSPHORUS AND MASS SPECTROMETRY
DETECTION**

PRINCIPLE

Basic drugs encompass the largest group of compounds analyzed at the OCME FTL. This procedure is designed to screen basic drugs in biological specimens using simultaneous analysis by gas chromatography (NPD) and full scan mass spectrometry (GCMS). The procedure is used for qualitative analysis.

Solid phase extraction (SPE) is used to extract basic drugs from biological fluids or tissue homogenates. In SPE, the drug is temporarily bound to a sorbent in the solid phase column as the prepared sample is poured through these columns. The column is washed to remove interfering compounds, followed by elution of the drug. The eluent is evaporated and then reconstituted. The resulting solution is analyzed by NPD/GCMS. A single point calibration curve is used for screening analysis along with a negative control (blank) and a 0.2 mg/L positive control. The multi-component calibrator and control contain analytes frequently encountered in this laboratory.

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 below. Each 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 (DI H₂O)**
2. **Negative Matrices**
 - a. 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.
4. **100mM sodium acetate buffer (pH 4.5)**
 - a. Dissolve 11.72g sodium acetate trihydrate in 400mL DI H₂O; add 6.48mL glacial acetic acid. Dilute to 2L using DI H₂O. Mix. Adjust pH to 4.5 ± 0.1 with sodium acetate or acetic acid. Note that this volume may be scaled up or down depending upon the needs of the laboratory.

- b. Storage: in glass; room temperature.
- c. Stability: 6 months; inspect daily before use for contamination.

5. **pH 9.0 buffer**

- a. Dissolve 40g KHCO_3 in 800mL DI H_2O ; add 20g K_2CO_3 . Dilute to 2L using DI H_2O . Mix. Adjust pH to 9.0 ± 0.1 with KHCO_3 to lower the pH, or K_2CO_3 to raise the pH.
- b. Storage: room temperature in glass.
- c. Stability: 6 months. Inspect for contamination each day of use.

6. **HCl, concentrated, 12.1N**. Fisher Scientific or equivalent.

7. **Hydrochloric Acid, 100mM**

- a. Add 16.8mL concentrated HCl to 800mL Deionized water. Dilute to 2000mL with deionized H_2O .
- b. Store at room temperature in glass or plastic.
- c. Stability: 6 months. Inspect each day before use for contamination.

8. **Methanol** (Fisher Scientific - ACS Certified)

9. **Ethyl Acetate**, Fisher Scientific Optima or equivalent.

10. **Ammonium Hydroxide, NH_4OH** (Fisher Scientific)

11. **Eluting solvent: Ethyl acetate/ NH_4OH (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 of ammonium hydroxide in a 6 mL crimp top vial. Use once then discard the remaining), open a fresh bottle daily. Always keep ammonium hydroxide refrigerated.

12. **Polycrom Clin II Solid Phase Extraction Columns**, CEREX

13. **System 48 Processor** connected to nitrogen source.

14. **Waste Rack, SPE Rack, Collection Tube Rack**.

15. **Concentrator (Turbovap or SPEware)** connected to a nitrogen source.

16. **Vacuum Filtration Apparatus**

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

- a. Add 780mL of toluene, 200mL of heptane and 20mL of isoamyl alcohol to a 1000mL graduated cylinder. Stir for approximately one hour, and then transfer to a 1L repipeter.

SPECIMEN PREPARATION

On occasion the FTL will receive specimens that may be of poor quality; these will be flagged as unsuitable for testing in both the appropriate case file and in DataEase. For example, decomposed specimens may exhibit the following characteristics: presence of green, brown, and black colors; layer(s) of fat; oily mixture in the sample and foul sample smell. Decomposition can affect results of testing so it is important to choose the most suitable specimen for testing. Characteristics of decomposed samples are not limited to what is listed above, the Medical Examiner may have noted that the specimen is decomposed and to what extent of this decomposition on the Toxicology Request form. If there is any question as to whether a sample is decomposed consult with a supervisor.

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

Ensure samples are homogenous; if necessary homogenize the specimen prior to aliquoting

Blood	1 mL of the undiluted specimen
Urine	1 mL of an undiluted specimen
Bile	1 mL of a 1:5 dilution (1 mL of undiluted bile qs to 5 mL with deionized water)
Brain	1 mL of a 1:3 homogenate (5 g homogenized with 10 mL of deionized water)
Liver	1 mL of a 1:5 homogenate (5 g homogenized with 20 mL of deionized water)
Gastric	1 mL of a 1:10 homogenate (For solid samples, homogenize 2 g with 18 mL of deionized water otherwise dilute 2 mL of thoroughly mixed gastric and q.s. to 20 mL.)

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.

EXTRACTION PROCEDURE

1. Print a worklist of cases to be analyzed. Review with supervisor for any updates of priority (RUSH) cases or other special instructions. Batches should contain **20-25 cases** plus the associated calibrator and controls. This batch size limit may be increased by

authority of the section supervisor as needed. 10% of the batch samples are to be QC specimens.

2. Retrieve all samples on the worklist and place in an empty rack. Allow samples to come to room temperature.

Note: If for any reason there is a discrepancy with the sample listed on the worklist for extraction check the appropriate case folder for any possible notations, if there is no information make a note in Data Ease, on the appropriate case analysis form, and ensure a discrepancy form was submitted for the sample. Consult with a supervisor as necessary.

i.e. sample listed on the worklist for extraction is subclavian blood but the sample submitted is not labeled or is labeled as another source

i.e. sample has low volume (QNS); may want to schedule a different sample for testing

3. Obtain enough 16 x 125 mm culture test tubes to extract one negative control, calibrator, positive controls and all requested case work. Label the tubes appropriately. Tubes should bear the entire toxicology number (e.g., 06-1234, **not** 1234), the specimen type and any appropriate dilution.
4. Pipet 1.0 mL of an appropriate negative matrix into each of the controls and calibrator tubes. Refer to the REAGENTS AND MATERIALS section of this document for specific matrix for the analysis.
5. Next, mix each specimen thoroughly by gently inverting container two to four times. Pipet 1.0 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. If a blood specimen is being pipetted and a clot is encountered, homogenize the specimen using either the Polytron Homogenizer or a glass hand homogenizer; document on the blood bottle that the specimen was homogenized, date and initial the label and annotate on sequence list. Continue in this fashion until all cases have been pipetted.

Note: *Immediately refrigerate all case specimens and negative matrices. Do not leave this for a later time.*

6. Remove all extracted cases in the DataEase database so the samples are not duplicated by another analyst.

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

7. Prepare calibrator by fortifying the appropriate tube with various solutions as indicated:
 - a. 1.0 mL blood aliquots:
Add 10 μ L of 100 mg/L Basic Drugs Group 1 Calibrator to the Cal 1 - 1.0mg/L tube only.
 - b. 1.0 mL urine aliquots:
Add 10 μ L of 100 mg/L Basic Drugs Group 1 Calibrator to the Cal 1 – 1.0mg/L tube only.

c. 1.0 mL tissue aliquots:

Add 10 µL of 100 mg/L Basic Drugs Group 1 Calibrator to the Cal 1 – 1.0mg/L tube only.

8. For **urine** screen batches prepare the 0.2 mg/L control (QC) by fortifying the QC 1 – 0.2 mg/L tube with 20 µL of 10 mg/L Basic Drugs Group 1 Control in 1.0 mL of deionized water.
9. For **blood** screen batches, obtain an appropriately labeled external 0.2 mg/L control (QAS) from the freezer and allow it to come to room temperature. Aliquot 1.0 mL of the QAS control into the appropriately labeled test tube.
10. For **tissue** screening, prepare the 0.2 mg/L control (QC) by fortifying the QC 1 – 0.2 mg/L tube with 20 µL of 10 mg/L Basic Drugs Group 1 Control in 1.0 mL of an appropriate negative matrix.
11. For **urine** samples, add 20 µL of 50 mg/L methapyrilene internal standard to all tubes (negative control, calibrator, QC, and cases).
12. For **blood** samples, add 20 µL of 50 mg/L methapyrilene internal standard to all tubes (negative control, calibrator, QC, and cases).
13. For **tissue** samples add 20 µL of 50 mg/L methapyrilene internal standard to all tubes (negative control, calibrator, QC, and cases).
14. Make note of the all the lot numbers of the standards used on the “Lot Sheet” form.
15. Vortex all tubes.
16. Add 6mL 100mM sodium acetate buffer pH 4.5 to the tubes. Vortex for 30 seconds and then sonicate for 15 minutes. Centrifuge sample for 15 minutes at ≈ 3000rpm.
17. Decant the supernatant into the appropriately labeled Polychrom Clin II column and apply nitrogen at a pressure of 2-4 psi. Ensure that each supernatant flows through the column; apply manual positive pressure as necessary.
18. Prepare the eluting solvent by pouring 98 mL of Ethyl Acetate into a glass flask; add 2mL of refrigerated NH₄OH. 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.

19. Wash Column (all wash steps are pressurized at 2-4psi). Ensure that all reagents have washed through the column; apply manual positive pressure as necessary.
 - 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 ≈ 25 psi (Max Flow)

20. Elute Drugs:

Place appropriately labeled 10mL conical centrifuge tubes under each column to collect eluate. Elute with 2mL eluting solvent (ethyl acetate/NH₄OH, 98/2); collect eluant at 1 to 2mL/minute. Ensure that all reagents have eluted through the column; apply pressure as necessary to achieve the desired flow rate.

21. Dry under nitrogen at room temperature to absolute dryness using a concentrator.
22. Reconstitute with 100 µL of THI. Mix by vortex and transfer to the glass insert in the appropriately labeled autosampler vial.
23. Immediately seal each vial with a screw-cap to avoid possible contamination from other samples. Do not wait until all transfers have been made to seal the vials. Samples may also be transferred into crimp cap vials and capped immediately. Transfer the vials onto the GC/MS Instrument and continue with analysis by gas chromatography/mass spectrometer/NPD.

INSTRUMENTATION

Agilent 6890 gas chromatograph with Agilent 5973 or 5975 mass spectrometer and a Agilent 6890 Series nitrogen-phosphorous detector equipped with a 7683A auto-sampler with controller and a RTX50 (Crossbonded 50% phenyl - 50% methyl polysiloxane) column (15m x 0.25mm x 0.25µm film thickness). A Dean splitter is used to split the output of the analytical column to the two detectors. A computer with Agilent Chemstation software is used to gather the raw data and Chemstation Enhanced Data Analysis software is used to process the data.

INSTRUMENT SETUP

6890 Series II Gas Chromatogram

1. The Dean splitter uses restrictors to control the split ratio. For all instruments, the restrictor is 0.25 mm ID deactivated fused silica. The approximate length of the restrictor from the Dean splitter to the MSD is 84 cm. The approximate length of the restrictor from the Dean splitter to the NPD detector is 27 cm.
2. Ensure that the selected instrument is operational and is not in need of maintenance. If maintenance is required, consult the appropriate manual and notify the supervisor.
3. Perform a Mass Spectrometric (MS) Tune to check if the instrument is operable. See the "Mass Spectrometric Tuning (Autotune)" SOP for specifications.
4. Check that the NPD bead voltage is set to current value as listed on the instrument.

Once the instrument is ready for use, load the screen method and setup a sequence. The Chemstation method file for screens is usually named DMSxEXPSCR.M, where the "x" refers to the instrument number. There should be a method file with the appropriate name in each method subdirectory. Consult supervisor if correct method is unclear.

DMSXEXPSCR.M (MS 6, AGILENT 5973)

METHOD SECTIONS TO RUN:

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

INSTRUMENT CONTROL PARAMETERS

Sample Inlet	GC
Injection Source	GC ALS
Mass Spectrometer	Enabled

HP6890 GC METHOD

OVEN

Initial temp	90°C (On)	Maximum temp	320 °C
Initial time	1.00 min	Equilibration time	0.50 min

<i>Ramps</i>			
#	Rate	Final temp	Final time
1	10.00	120 °C	1.00
2	20.00	295	8.25
3 (off)	295°C		
Post temp	295°C		
Post time	3.00 min		
Run time	22.00 min		

INLET

<i>Front Inlet (Unknown)</i>	
Mode	Splitless
Initial temp	260 °C (On)
Pressure	11.39 psi (On)
Purge Flow	30.0 mL/min
Purge Time	2.00 min
Total flow	33.9 mL/min
Gas saver	On
Saver Flow	20.0 mL/min
Saver Time	3.50 min
Gas type	Helium

<i>rear Inlet (epc for dean splitter)</i>	
Mode	Sim dist
Initial temp	
Pressure	4.0 psi (On)
Purge Flow	
Purge Time	
Total flow	
Gas saver	
Saver Flow	
Saver Time	
Gas type	Helium

COLUMN

<i>Column 1</i>		<i>Column 2</i>
Capillary Column		(not installed)
Model Number:	Restek RTX-50	
Max temperature	320 °C	
Nominal length	15.0 m	
Nominal diameter	250.00 µm	
Nominal film thickness	0.25 µm	
Mode	Ramped flow	
Initial flow	1.5 mL/min	
Initial Time	13.00 min	
# Rate Final Flow Final Time		
1 1.00 3.5 8.25		
2 0.0 (off)		
Post Flow:	4.0 mL/min	
Nominal initial pressure	13.58 psi	
Average velocity	38 cm/sec	
Inlet	Front Inlet	
Outlet	MSD	
Outlet pressure	4.00 psi	

DETECTOR

<i>FRONT DETECTOR (NPD)</i>		<i>BACK DETECTOR (NO DET)</i>
Temperature	325 °C	<i>(No detector installed)</i>
Hydrogen flow	3.0 mL/min	
Air flow	60.0 mL/min	
Mode	Constant makeup flow	
Makeup flow	29.0 mL/min	
Makeup Gas	Helium	
Adjust Offset	30.0	
Electrometer	On	
Bead	On	
Equilibration Time	5.00 min	

SIGNAL

<i>Signal 1</i>		<i>Signal 2</i>
Data rate	20 Hz	<i>(not set)</i>
Type	front detector	
Save Data	On	
Start Save Time	3.00 min	
Stop Save Time	22.00 min	
Zero	0.0 (off)	
Range	0	
Fast Peaks	Off	
Attenuation	0	

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	3.00 min
Oven Temperature	295 °C
Column 1 flow	4.0 mL/min
Column 2 Pressure	0.0 psi

7673 INJECTOR

<i>Front Injector:</i>	
Sample Washes	1
Sample Pumps	4
Injection Volume	3.0 microliters
Syringe Size	10.0 microliters
PreInj Solvent A Washes	1
PreInj Solvent B Washes	1
PostInj Solvent A Washes	3
PostInj Solvent B Washes	3
Viscosity Delay	0 seconds
Plunger Speed	Fast
PreInjection Dwell	0.00 minutes
PostInjection Dwell	0.00 minutes

Back Injector:

No parameters specified

MS ACQUISITION PARAMETERS

General Information	
Tune File	atune.m
Acquisition Mode	Scan
MS Information	
Solvent Delay	3.50 min
EMV Mode	Relative
Relative Voltage	0.0
Resulting EM Voltage	(varies)
Scan Parameters	
Low Mass	35.0
High Mass	550.0
Threshold	150
Sample #	2 A/D Samples 4

Plot 2 low mass	35.0
Plot 2 high mass	550.0
MS Zones	
MS Source	230°C (max 250 °C)
MS Quad	150°C (max 200 °C)

DMSXEXPSCR.M (MS 7, AGILENT 5973)

METHOD SECTIONS TO RUN:

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

INSTRUMENT CONTROL PARAMETERS

Sample Inlet	GC
Injection Source	GC ALS
Mass Spectrometer	Enabled

HP6890 GC METHOD

OVEN

Initial temp	90°C (On)	Maximum temp	320 °C
Initial time	1.00 min	Equilibration time	0.50 min

<i>Ramps</i>			
#	Rate	Final temp	Final time
1	10.00	120 °C	1.00
2	20.00	295	8.25
3 (off)	295°C		
Post temp	295°C		
Post time	3.00 min		
Run time	22.00 min		

INLET

<i>Front Inlet (Unknown)</i>	
Mode	Pulsed Splitless
Initial temp	260 °C (On)
Pressure	5.08 psi (On)
Pulse Pressure	25.0 psi
Pulse time	0.75 min
Purge Flow	40.0 mL/min

Purge Time	2.00 min
Total flow	44.2 mL/min
Gas saver	On
Saver Flow	20.0 mL/min
Saver Time	3.50 min
Gas type	Helium

<i>rear Inlet (epc for dean splitter)</i>	
Mode	Sim dist
Initial temp	
Pressure	4.0 psi (On)
Purge Flow	
Purge Time	
Total flow	
Gas saver	
Saver Flow	
Saver Time	
Gas type	Helium

COLUMN

<i>Column 1</i>		<i>Column 2</i>
Capillary Column		(not installed)
Model Number:	Restek RTX-50	
Max temperature	320 °C	
Nominal length	15.0 m	
Nominal diameter	250.00 µm	
Nominal film thickness	0.25 µm	
Mode	Ramped flow	
Initial flow	1.3 mL/min	
Initial Time	13.00 min	
# Rate Final Flow Final Time		
1 1.50 4.0 4.0		
2 0.0 (off)		
Post Flow:	4.0 mL/min	
Nominal initial pressure	5.08 psi	
Average velocity	60 cm/sec	
Inlet	Front Inlet	
Outlet	MSD	
Outlet pressure	4.00 psi	

DETECTOR

<i>FRONT DETECTOR (NPD)</i>		<i>BACK DETECTOR (NO DET)</i>
Temperature	325 °C	<i>(No detector installed)</i>
Hydrogen flow	3.0 mL/min	
Air flow	60.0 mL/min	
Mode	Constant makeup flow	
Makeup flow	30.0 mL/min (On)	

Makeup Gas	Helium
Adjust Offset	30.0
Electrometer	On
Bead	On
Equilibration Time	5.00 min

SIGNAL

<i>Signal 1</i>		<i>Signal 2</i>
Data rate	20 Hz	(not set)
Type	front detector	
Save Data	On	
Start Save Time	3.82 min	
Stop Save Time	22.00 min	
Zero	0.0 (off)	
Range	0	
Fast Peaks	Off	
Attenuation	0	

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	3.00 min
Oven Temperature	295 °C
Column 1 flow	4.0 mL/min
Column 2 Pressure	0.0 psi

7673 INJECTOR

<i>Front Injector:</i>	
Sample Washes	1
Sample Pumps	4
Injection Volume	3.0 microliters
Syringe Size	10.0 microliters
PreInj Solvent A Washes	1
PreInj Solvent B Washes	1
PostInj Solvent A Washes	3
PostInj Solvent B Washes	3
Viscosity Delay	0 seconds
Plunger Speed	Fast
PreInjection Dwell	0.00 minutes
PostInjection Dwell	0.00 minutes

Back Injector:
No parameters specified

MS ACQUISITION PARAMETERS

General Information	
Tune File	atune.m
Acquisition Mode	Scan
MS Information	
Solvent Delay	3.00 min
EMV Mode	Relative
Relative Voltage	0.0
Resulting EM Voltage	Varies
Scan Parameters	
Low Mass	35.0
High Mass	550.0
Threshold	150
Sample #	2 A/D Samples 4
Plot 2 low mass	35.0
Plot 2 high mass	550.0
MS Zones	
MS Source	230°C (max 250 °C)
MS Quad	150°C (max 200 °C)

DMS9EXPSCR.M (MS9, AGILENT 5975)

METHOD SECTIONS TO RUN:

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

INSTRUMENT CONTROL PARAMETERS

Sample Inlet	GC
Injection Source	GC ALS
Mass Spectrometer	Enabled

HP6890 GC METHOD

OVEN

Initial temp	90°C (On)	Maximum temp	320 °C
Initial time	1.00 min	Equilibration time	0.50 min

<i>Ramps</i>			
#	Rate	Final temp	Final time
1	10.00	120 °C	1.00

2	20.00	295	10.25
3 (off)	0.0		
Post temp	295°C		
Post time	3.00 min		
Run time	24.00 min		

INLET

<i>Front Inlet (Unknown)</i>	
Mode	Pulsed Splitless
Initial temp	260 °C (On)
Pressure	5.08 psi (On)
Pulse Pressure	25.0 psi
Pulse time	0.75 min
Purge Flow	40.0 mL/min
Purge Time	2.00 min
Total flow	44.2 mL/min
Gas saver	On
Saver Flow	20.0 mL/min
Saver Time	3.50 min
Gas type	Helium

<i>rear Inlet (epc for dean splitter)</i>	
Mode	Sim dist
Initial temp	
Pressure	0.0 psi (off)
Purge Flow	
Purge Time	
Total flow	
Gas saver	
Saver Flow	
Saver Time	
Gas type	Helium

COLUMN

<i>Column 1</i>		<i>Column 2</i>
Capillary Column		(not installed)
Model Number:	Restek RTX-50	
Max temperature	320 °C	
Nominal length	15.0 m	
Nominal diameter	250.00 µm	
Nominal film thickness	0.25 µm	
Mode	Ramped flow	
Initial flow	1.5 mL/min	
Initial Time	13.00 min	
# Rate Final Flow Final Time		

1	0.55	3.0	5.0		
2	0.0 (off)				
Post Flow:				4.0 mL/min	
Nominal initial pressure				6.65 psi	
Average velocity				64 cm/sec	
Inlet				Front Inlet	
Outlet				MSD	
Outlet pressure				Vacuum	

DETECTOR

<i>FRONT DETECTOR (NPD)</i>		<i>BACK DETECTOR (NO DET)</i>
Temperature	325 °C	<i>(No detector installed)</i>
Hydrogen flow	3.0 mL/min	
Air flow	60.0 mL/min	
Mode	Constant makeup flow	
Combined flow	30.0 mL/min	
Makeup flow	On	
Makeup Gas	Helium	
Adjust Offset	30.0	
Electrometer	On	
Bead	On	
Equilibration Time	5.00 min	

SIGNAL

<i>Signal 1</i>		<i>Signal 2</i>
Data rate	20 Hz	<i>(not set)</i>
Type	front detector	
Save Data	On	
Start Save Time	2.50 min	
Stop Save Time	24.00 min	
Zero	2.6 (On)	
Range	0	
Fast Peaks	Off	
Attenuation	0	

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)

AUX PRESSURE 3

description	Controls Dean splitter
Gas type	helium
Initial pressure	3.8 psi

POST RUN

Post Time	5.00 min
Oven Temperature	295 °C
Column 1 flow	1.2 mL/min

7673 INJECTOR

<i>Front Injector:</i>	
Sample Washes	1
Sample Pumps	2
Injection Volume	3.0 microliters
Syringe Size	10.0 microliters
PreInj Solvent A Washes	1
PreInj Solvent B Washes	1
PostInj Solvent A Washes	3
PostInj Solvent B Washes	3
Viscosity Delay	0 seconds
Plunger Speed	Fast
PreInjection Dwell	0.00 minutes
PostInjection Dwell	0.00 minutes

Back Injector:

No parameters specified

MS ACQUISITION PARAMETERS

General Information	
Tune File	atune.m
Acquisition Mode	Scan
MS Information	
Solvent Delay	3.80 min
EMV Mode	Gain Factor
Gain Factor	1.00
Resulting EM Voltage	(varies)
Scan Parameters	
Low Mass	35.0
High Mass	550.0
Threshold	150
Sample #	2
Plot 2 low mass	50.0
Plot 2 high mass	550.0
MS Zones	
MS Source	230°C (max 250 °C)
MS Quad	150°C (max 200 °C)

SEQUENCE

When Chemstation is opened, the **Openlab ECM Login** screen appears. Enter the instrument name as appropriate for username and the current password. Verify that Account field is

production and Domain field is Built-In. If Chemstation is already running, it may be necessary to log out and log in again. Using the Chemstation software, at the top toolbar under **ECM**, select **Logon to ECM**. Follow the instructions above to log on.

1. On the Method and Run toolbar, under **Sequence**, select **Load Sequence**. Select **default.s**. Click on Select.
2. In the Method and Run toolbar, under **Sequence**, select **Edit Sequence**. At the top of the screen under Data Path, click on Browse. Under **Select Data Path**, click on the **msdchem** folder. The click **1** and then click on the **Data** folder to highlight it. In lower left of dialog box, select **Make New Folder**. A folder with the name New Folder is created under DATA. Right click on **New Folder** and **Rename** or double click to highlight the folder name and change the entry. Rename the file using the format MSNMMDDYYx, where N is instrument number, MM = month, DD = day, YY= year and x = a letter indicating the batch being run, e.g. MS3041111a. Click **OK** which will take you back to the sample log table.
3. In the Sample column verify the correctness of pre-loaded entries. Starting at the first empty field, enter sample or QC information. For samples this would include aliquot number, laboratory number, specimen source, dilution if any (i.e., 2-11-2432 fem). If the sample has been diluted, enter the appropriate dilution. For QC samples use an appropriate designator. Enter the next sample in the batch in the next open field down the column.
4. In the Type column, select the corresponding sample type for each vial: Sample, Blank, Calibration or QC.
5. In the Vial column, click in the cell with the number 1, hold down the left mouse button and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column and Increment. Verify that the vial numbers are correct.
6. In the Method column, verify that the correct method is loaded in the first cell. Then click on the first cell containing the method name, hold down the left mouse and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column, No Increment. Verify that the method for each vial is correct.
7. In the Data File column, in the cell corresponding to the first vial, enter the data file name in the format MSNMMDDx001, where N = instrument number, MM = month, DD = day, and x = a letter indicating the batch being run, i.e. MS20411a001a. Click on this cell, hold down the left mouse and drag to the last vial number of the sequence (the cells will be highlighted). Right click and select Fill Column and Increment. Verify that the data file information for each vial is correct.
8. In the Comment column, enter any additional information for the vials.
9. In the Multiplier column, enter any sample dilutions.
10. In the **Level** column, verify that the correct level number (1) is entered for calibrator 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**.
13. Go to **Sequence** → **Simulate Sequence** → **Run Sequence**.

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.

A 2nd dialog box will pop-up: Create C:\MSDCHEM\1\DATA\MSNMMDDYYy? → Click Yes.

A 3rd dialog box will pop-up: Sequence Verification Done! View it? → Click Yes or No.

14. Save sequence accordingly.

15. 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., WAD). Under **Data File Directory** verify that the data file path is C:\MSDCHEM\1\DATA\batch name.

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

Note: Each sequence/subsequence **MUST** end with a positive control (it may be a re-injection of a previously injected positive QC).

Setting-Up a Subsequence using the Same Method

On the Sample Log Table:

- a. Select "**Keyword**" for "Type"
- b. Select "**DataPath**" for "Method/Keyword"
- c. Under "Comment/KeywordString" type in the *new data path* for your subsequence i.e.,: **C:\MSDCHEM\1\DATA\MSNMMDDYYx** where "N" is the instrument's number and "x" is the letter designated to the subsequence (**it must be different than that of the original sequence**).
- d. The suffix of the Data Files must be different from that of the original; i.e.,: MSNMMDDy001; **the subsequence data files must start with 1 again; Click OK.**
- e. Go to **Sequence** → Simulate Sequence → **Run Sequence**.

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.

A 2nd dialog box will pop-up: Create **C:\MSDCHEM\1\DATA\MSNMMDDYYy**? → Click **Yes**.

A 3rd dialog box will pop-up: Sequence Verification Done! View it? → Click **Yes** or **No**.

- f. 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., WAD). Under **Data File Directory** verify that the data file path is C:\MSDCHEM\1\DATA\batch name.

Setting-Up a Subsequence with a Different Method

On the Sample Log Table:

- a. After inserting the DataPath keywords and Commands, Insert a Row.

- b. Select "**Keyword**" for "Type"
- c. Select "**MethodPath**" for "Method/Keyword"
- d. Check that the method for each sample is changed to the new method.
- e. Go to **Sequence** → Simulate Sequence → **Run Sequence**.

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.

A 2nd dialog box will pop-up: Create **C:\MSDCHEM\1\DATA\MSNMMDDYYy?** → Click **Yes**.

A 3rd dialog box will pop-up: Sequence Verification Done! View it? → Click **Yes** or **No**.

- f. 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., WAD). Under **Data File Directory** verify that the data file path is C:\MSDCHEM\1\DATA\batch name.
- g. 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.

THE FOLLOWING APPLIES TO ALL SEQUENCE SET-UPS (i.e. sequence, sequence with same method, or sequence with a different method):

1. 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.
2. 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.
3. 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**.
4. 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.

SAVE METHOD TO ECM

1. After the run finishes, the raw 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.

DATA TRANSFER AND PROCESSING

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

LOAD METHOD AND BATCH – EXPORTING RAW DATA FOR PROCESSING

1. On the left screen under the C drive, open C:\msdchem\1\ecm\retrieve
2. Under retrieve, click on the batch that will be 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.

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 Batch From ECM. Browse to locate and select the batch to be processed. When the proper batch is highlighted, click OK to start the retrieve. Each data file will be retrieved along with the method to be used for processing. Verify that the sequence and method are the correct files that will need to be processed.
3. Once the retrieval is complete the batch folder is located in the C:\msdchem\1\DATA\ECM\retrieve folder.

4. Select the **Method** under the batch by right clicking on it and Load the method. Select the Calibrator 1 data file in the batch (usually the first one), right click, and select Load to load the data into memory.
5. In the white area just below the bottom icon bar, type QT 1, then click the execute button to the right of the white area. This will quantitate the data using the loaded method, but will not produce a report.
6. Next click on the QEDIT icon (or under view in the menu bar, select QEDIT Quant Result). Four windows will be presented. On the far left, a list of all compounds in the calibration table starting with the mass spec compounds, then the NPD compounds; located center and right on top are the extracted ions of the compound selected; located center bottom are the spectra of the selected peak; and located bottom right is information about the compound selected from the list to the left.
7. Click on custom tool 5 icon (hammer with a 5 next to it, last icon on the first row of icons). This removes any compound that is marked as present (indicated as a star in the compound list) but does not have a compound type of A in the method table. This macro must be used with caution. It is safe to use with a calibrator or control for calibrator 1, as only the compounds in the calibrator have A in the compound type.
8. Double click on the internal standard (methapyrilene), the first compound in the list. Review the integrated baseline to confirm that the correct peak for the compound has been selected and the extracted ion chromatographs were properly integrated.

NOTE: *Peak shape should be Gaussian or symmetrical. Redraw baselines if necessary. If the wrong peak was selected, draw baselines under the correct peak in the ion chromatographs.*

- a. Click on custom tool 1 (**hammer 1**). This macro toggles back and forth between the mass spec compound and the corresponding NPD compound. The compound name in the NPD results shows the NPD chromatogram in place of the extracted ion chromatographs shown previously. Review as above, checking that the peak assignment and baseline is correct. Make any necessary corrections.

NOTE: If the compound has been misidentified by the software, click the Qdel button to mark the compound as not detected. Double right clicking in the mass spectrum window will perform a library search of the spectrum displayed. Use as necessary to check peak identity. Double clicking on the compound names marked with a star will load that compound for review.

Repeat steps 7-8 above for each compound in the calibrator following the same listed criteria. When finished, click exit. A window will appear. Select yes to "save changes to quantitative results?" If no changes to the compounds were made, it may be appropriate to select no.

9. When all compounds in the calibrator have been reviewed, click on the Update Calibration icon (graph with yellow up arrow, in the middle right of the second row of icons) to update the single level of calibration. A window appears. Make sure "Update one level" is selected, and click OK. A window appears, saying "file has previously been quantitated. Requantitate now?". Select no. Then the Update Calibration window appears. Click on "Update Level (select existing Calibration Level ID)", so that a black dot appears in the white circle. Then select Responses, Replace, Retention Times, Replace and Replace Qualifier Relative Responses. Highlight 1 in the Existing Level ID box. Click on Do Update button. This will update the calibration for level 1 based on the calibrator just reviewed.

10. The Edit Compounds screen appears. Click on OK. A window appears asking “current changes will be saved. Proceed?”. Click OK. This produces a calibrated method in memory
11. In the menu bar select **Method**. Select save method. A box appears, showing the path to the method. Check to make sure the method path is correct, leading to the method under the current batch that is being processed. Another box appears, with a browser in ECM. Click cancel, as the method will be saved to ECM later, when printing is completed, and after all changes are made.
12. Now all the files in the batch being processed must be quantitated against the calibrated method. On the menu toolbar, click on Tools and then click on Dolist. The Qlist Configuration window will appear. Under the Qlist Configuration window. In the left hand window are various options. In the Selected Options window on the right, the only program listed should be Quant No Report (QT 1). If any other option(s) is present, click to highlight it and select remove. If Quant No Report is not present in the Selected Options box, choose Quant No Report from the left side by clicking on it and then select Add. Verify that when Quant No Report is highlighted in the left side box, the command string line at the bottom of the window shows QT 1. Finish by selecting OK.
13. Under the Multiple File screen, verify that the correct batch is loaded. If the correct batch is not loaded, load the correct batch by selecting Change Path, then selecting the batch to be loaded. Select OK. Verify that the path for the data files is correct.

Note: Correct path is C:\msdchem\ecm\retrieve\current batch name

14. In the left “available data file” screen, highlight all files and select right arrow to load them into the Files Selected for Processing window. Click Process. The entire batch will be quantitated.
15. To review the processed data files on the “Data Analysis” screen, select the data file (in this case, Calibrator 1 data file) in the batch, right click, and select Load to load the data into memory.
16. Visually examine the chromatographs for significant peaks, especially peaks that may not be compounds in calibrator 1. Note any peaks that are shown in the TIC, but do not show in the NPD chromatogram, indicating that the peak is not nitrogenous. By right double clicking on the apex of a peak, the mass spectrum of that point will be shown in a window. Right double clicking on a point at the leading edge of the peak will load that spectrum. By clicking on the subtract icon (11th from the left in the second row of icons), a background-subtracted mass spectrum is displayed. This spectrum may be library searched by right double clicking in the spectrum window. The libraries searched and other criteria can be adjusted by clicking on the icon immediately to the right of the subtract icon.
17. Click on the QEDIT icon (or under view in the menu bar, select QEDIT Quant Result). Four windows will be presented. On the far left, a list of all compounds in the calibration table starting with the mass spec compounds, then the NPD compounds; located center and right on top are the extracted ions of the compound selected; located center bottom are the spectra of the selected peak; and located bottom right is information about the compound selected from the list to the left. When reviewing case samples, use custom tool 5 with great caution. Sometimes it is helpful to delete all but “A” compounds and then reintegrate the compounds found when reviewing the chromatographs outside of Qedit.

18. Repeat steps 17 and 18 above for each compound marked with a star, and for any compounds identified in the chromatogram review step, until all compounds present have been identified (marked with a star in the Qedit list). When finished, click exit. A window will appear. Select yes to “save changes to quantitative results?”.
 19. On the menu bar, click Tentatively ID, then LSC Summary to Screen. This will integrate the TIC, subtract any peaks marked as identified in Qedit, select the 15 largest peaks remaining, perform a background subtract, and then perform a library search on each peak. When this is done, click Tentatively ID, then Edit LSC results. a window will appear, showing the mass spectrum of the first peak, and the library spectrum of the best hit. Compare the two spectra. If there is a match, click next, and the next spectrum will be displayed. If there is no match, click delete, then next.
 20. Click on the next data file icon (second from left, first row of icons) to load the next data file.
 21. Repeat steps 16 through 20 above for each data file in the batch.
 22. Save files to ECM. Select ECM from the toolbar, select “Save multiple data files to ECM”. Select all files.
 23. Save method to ECM. Select ECM from the toolbar, Save Method to ECM. Make sure data path is correct.
 24. To print reports, select Tools from the toolbar, DoLIST, full quant w/o calculations (QT 0,1,`F`), and detailed LSC w/o calculations (Report_TIC 0,1,`F`), Add, and OK. Select files to print, click the → Arrow and Process.
- NOTE:** If for any reason re-analysis is performed on the processed data (i.e. baseline edited, peak deleted, peak identified that was not previously identified, etc.) a copy of the processed data file should be created; the saved file will be termed “Version 2”.
25. Submit report for Second Level Review.

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.

CARRYOVER

1. Two types of carryover are possible with the methods employed by GC/MS. Carryover of late eluting peaks from one injection into one or more subsequent injections is usually apparent from the shape of the peak (broad). The likelihood of this type of carryover is decreased by the extended length of the MS. Although the MS is used in a qualitative screen mode, this type of carryover can be determined by the ion pattern and the expected relative retention time of the corresponding analyte (if any library matches are made). If this phenomenon is observed, re-inject the sample with the suspected carryover, and inject the sample that was injected just before the carryover sample on a

GCMS using a longer high temperature program. Annotate on the file folder that the re-injections have been scheduled.

2. The other possibility for a carryover is contamination from an excessive amount of the analyte in the previous sample, either through the syringe or by drug being retained in the injection port or on the column. All positive samples are quantitated in their appropriate department(s) following extraction of a new aliquot. Discrepant results from these two extractions require a third aliquot to be extracted. Frequent changing of the solvent in the autosampler wash vials helps to control this type of potential carryover.

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. Significant peaks have been properly identified, integrated and labeled, review the chromatograms to make sure that peaks have been identified and integrated, peak shape should be Gaussian or symmetrical, .

Note: To determine whether peaks have been properly identified and integrated, the processed data is readily accessible and can be viewed in ChemStation software by the second reviewer should additional analysis of the data be necessary. In addition, raw data is always available and readily accessible and can be viewed in ChemStation software if re-analysis is necessary.

2. Verify that all components of the calibrator and controls are present and that each peak is properly assigned. Correct any errors. If any compounds do not extract, or if multiple peaks are detected for a compound in the calibrator or control, the analyst must consult with a supervisor.
3. Check the blank for any 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 is ANY question.
4. 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.
5. The retention time for a peak must be within $\pm 2\%$ of the calibrators or controls, peak base ion and two additional ions must be present and compared to those from software libraries. The quality % match and internal standard recovery should be within acceptable criteria (see Acceptance Criteria).
6. 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 or poor recovery.

REPORTING

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

Report using the following guidelines:

1. Include a copy of the sequence and the original tune report in the instrument logbook.
2. Each case printout must have a copy of the control packet; this includes sequence, calibrators, all controls and lot form sheet.
3. Review the printouts, and report the results as “(drug X) detected”, or, if none were detected, as “Basic Drugs Not Detected” (NDT).

NOTE: In certain instances a standard may not be attainable; therefore it is called “Detected by MS Match Only”.

4. All samples with preliminary positive results are scheduled for confirmatory analysis and/or quantitation as needed.
5. Submit the case file with all reports for Third Level Review.

NOTE: If the case history indicates a particular analyte is likely to be found and the analyte is not detected check the processed data at specified retention time location to ensure all peaks were identified and integrated properly. If a peak is a presumptive analyte, additional analysis may be scheduled as follows:

- a. reinjection with a higher volume
- b. reinjection of sample after performing instrument maintenance and/or use a different instrument
- c. extract the same specimen a second time using dilutions or add more buffer, or extract a different specimen.

NOTE: If a sample is sent back for reanalysis, a note should be made on the corresponding chromatogram indicating such. If reanalysis yields the same results as the initial analyses, then the analyte in question cannot be conclusively reported.

NOTE: If a sample or chromatogram is deemed unsuitable a note will be made to the appropriate case in DataEase, a comment will be made on the “Forensic Toxicology-Case Analysis” form which will be placed in the case file.

SCHEDULING ADDITIONAL TESTING (QUANTITATIVE OR REPEAT ANALYSIS)

In the Toxicology DataEase, choose the appropriate screen(s) for the drugs identified. In addition, document the necessary scheduling on the case analysis form in the corresponding case file.

The following scheduling guidelines must be followed.

- a. Blood testing scheduled from urine screen results:

1. Confirm type of blood specimen to be scheduled.
2. Assign 1 (one) as the aliquot number.
3. Fill in the appropriate fields for the case analysis form being used.

Note: *When scheduling cases for blood testing containing methadone metabolite enter "Yes" in the "Methadone/MM?" field of the "New Blood/Tissue Quant" screen in DataEase.*

- b. Blood testing scheduled from blood screen results:
 1. Confirm type of specimen needed for scheduling.
 2. Use the next sequential aliquot number, e.g. if the screen data is from aliquot 2, the next aliquot number will be 3.
 3. Fill in the appropriate fields for the case analysis form being used.
 4. Note dilutions (if needed) in comment field.
- c. Tissues are scheduled for quantitation at the discretion of the reviewer at the time of final review or at the request of the medical examiner of record.

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.

ACCEPTANCE CRITERIA – APPLIES TO ALL LEVELS OF REVIEW

1. External or internal controls must be analyzed according to 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 are detected in the negative control (blank).
3. The positive control must contain each analyte as an integrated peak. Since this is a screening procedure, the control does not need to quantitate to the target concentration, since no quantitative data is used from the qualitative analysis.
4. The analytes must be detected and pass the requirements for acceptable chromatography. Although the quantitative data from screens is not to be reported, it may be used as an approximation for deciding analytical strategy for quantitation, and for comparison with quantitative results.

5. Check chromatography of all injections. Examine the peak shape and evaluate any peaks that show non-Gaussian shape. Identify unresolved peaks and peaks with shoulders on either side. Consult a supervisor about any unusual events.
6. Check that all significant peaks in the chromatogram are integrated. Check if the baseline used to integrate is appropriate.
7. Criteria for full scan are based on comparison of full scan data for the case with reference scan data contained in system libraries.
8. Base ion must be present.
9. Two additional significant ions must be present (these may be the ions designated in the method or additional known ions from reference materials or libraries.)
10. Retention times of target analytes should match those of the calibrator or controls within $\pm 2\%$ of the analyte corresponding retention time
11. The mass spectra of the peak in question must match the mass spectra of the reference or library scan. Most of the significant ions in the reference scan should be present in the sample scan. Any doubts regarding the correct identification, due to the complex nature of mass spectra, should be discussed with a supervisor.

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REVISION HISTORY

- | | |
|------------|---|
| 04.01.2013 | 1. Revision history implemented. |
| 04.01.2015 | 1. Increased volume of stock buffer solutions.
2. Included an end of run QC sample.
3. Minor grammar changes. |
| 09.02.2015 | 1. Minor grammar changes.
2. Redefined levels of review. |