

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

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

PRINCIPLE

This method is applied to the quantitation of amphetamine (A), methamphetamine (MA), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), ephedrine (EPHD), pseudoephedrine (PSEPHD), phentermine (PHENT), fenfluramine (FEN), methylenedioxyethylamphetamine (MDEA), phenylpropanolamine (PPA) and para-methoxyamphetamine (PMA). It is also applied to the quantitation of buphedrone, mephedrone, methylone and Methylenedioxypropylvalerone (MDPV) the so called “bath salts”.

Sympathomimetic amines and “bath salts” are extracted from biological specimens using solid phase extraction. Drugs are temporarily bound to a sorbent in the solid phase cartridge as the prepared sample is poured through the column. The column is washed to remove interfering compounds, followed by the elution of drugs from the column using an organic solvent. The eluate is evaporated and the residue containing the drugs is derivatized using trifluoroacetic anhydride (TFAA). The trifluoroacetylated derivatives are extracted in order to remove the excess derivatizing reagent.

Quantitative analysis is performed by SIM GCMS using a four point calibration curve with deuterated internal standards.

SAFETY

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

SPECIMEN PREPARATION

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

Blood	1.0 mL of the undiluted specimen
Urine	1.0 mL for qualitative identification
Brain	1.0 mL of a 1:3 homogenate
Gastric Contents	1.0 mL of a 1:10 dilution
Liver	1.0 mL of a 1:5 homogenate

Vitreous Humor	1.0 mL of the undiluted specimen
Bile	1.0 mL of the undiluted specimen

Dilution of specimens

Specimens are diluted as follows:

Brain 1:3	5.0 g of brain homogenized with 10 mL of distilled water.
Liver 1:5	5.0 g of liver homogenized with 20 mL of distilled water.
Gastric 1:10	2.0 mL of liquid <i>q.s.</i> to 20 mL of distilled water, or 2.0 g of a solid specimen homogenized with 18 mL of distilled water.

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

REAGENTS AND MATERIALS

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** (distilled can be substituted)
2. **Methanol** (Fisher Scientific - ACS Certified)
3. **Ammonium Hydroxide, NH₄OH** (Fisher Scientific)

Note: Ammonium hydroxide will break down to ammonia and water and the ammonia will evaporate if the container is not kept closed. This will cause the 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). Open a fresh bottle daily, use once then discard the remaining. Always keep ammonium hydroxide refrigerated.

4. **Ethyl Acetate.** Fisher Scientific or equivalent.
5. **Methylene Chloride.** Fisher Scientific or equivalent.
6. **2-Propanol.** Fisher Scientific or equivalent.
7. **Sodium Phosphate Monobasic.** Certified A C S. Fisher Scientific or equivalent.
8. **Sodium Phosphate Dibasic.** Certified A C S. Fisher Scientific or equivalent.
9. **Certified Negative Blood.** Fortified with NaF. Previously found to have no interfering peaks. Freeze validated negative blood. Discard after 3 months.
10. **Certified Negative Liver.** Previously found to have no interfering peaks. Freeze validated negative liver. Discard after 3 months.

11. **Certified Negative Brain.** Previously found to have no interfering peaks. Freeze validated negative brain. Discard after 3 months.
12. **Polycrom Clin II Solid Phase Extraction Column.** CEREX
13. **System 48 Processor** connected to nitrogen source.
14. **Waste Rack, SPE Rack, Collection Tube Rack.**
15. **Turbovap** connected to a nitrogen source.
16. **Sonicator**
17. **Vortex**
18. **Centrifuge**
19. **100 mM phosphate buffer (pH 6.0)**
Dissolve 3.40 g Na_2HPO_4 and 24.28 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 1000 mL DI H_2O .

Dilute to 2000 mL using DI H_2O . Mix. Adjust pH to 6.0 + 0.1 with monobasic sodium phosphate (lowers pH) or dibasic sodium phosphate (raises pH).

Stability: 1 month. Inspect each day of use contamination.
20. Eluting solvent: CH_2Cl_2 /IPA/ NH_4OH (78/20/2). Prepare fresh each day of use.
21. 1% methanolic HCl.
22. Trifluoroacetic anhydride (TFAA). Aldrich Chemical.
23. Toluene. Fisher Scientific or equivalent.
24. Primary Standards: solutions in methanol, Cerilliant or equivalent, purchased at stated concentration in solution.

Amphetamine	1 mg/mL in methanol
Fenfluramine	1 mg/mL in methanol
Methamphetamine	1 mg/mL in methanol
Methylenedioxyamphetamine	1 mg/mL in methanol
Methylenedioxymethamphetamine	1 mg/mL in methanol
Ephedrine	1 mg/mL in methanol
Phenylpropylamine	1 mg/mL in methanol
Paramethoxyamphetamine	1 mg/mL in methanol
Phentermine	1 mg/mL in methanol
Pseudoephedrine	1 mg/mL in methanol
Methylenedioxyethylamphetamine	1 mg/mL in methanol
Buphedrone	1 mg/mL in methanol
Mephedrone	1 mg/mL in methanol
Methylone	1 mg/mL in methanol
Methylenedioxypropylamphetamine	1 mg/mL in methanol

25. Calibrators and controls

Calibrators and in-house controls are prepared according to the section "Preparation of GC Calibrators and Controls" for the following drugs used in Groups 1-3.

Group 1	Group 2	Group 3
Amphetamine	Ephedrine	Buphedrone
Methamphetamine	Fenfluramine	Mephedrone
MDA	MDEA	Methylone
MDMA	Phentermine	Methylenedioxypyrovalerone
Phenylpropanolamine	PMA	
	Pseudoephedrine	

A. 100 mg/L Working Calibrator and Control

Group 1 - pipet 5 mL of 1 mg/mL primary standard solution (or 5 x 1.0 mL from ampoules) of Amphetamine, Methamphetamine, MDA, MDMA and Phenylpropanolamine to a 50 mL volumetric flask. Dilute to mark with methanol. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

Group 2 - pipet 5 mL of 1 mg/mL stock solution (or 5 x 1.0 mL from ampoules) of Ephedrine, Fenfluramine, MDEA, Phentermine, PMA and Pseudoephedrine to a 50 mL volumetric flask. Dilute to mark with methanol. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

Group 3 - pipet 5 mL of 1 mg/mL stock solution (or 5 x 1.0 mL from ampoules) of Buphedrone, Mephedrone, Methylone and MDPV to a 50 mL volumetric flask. Dilute to mark with methanol. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

Repeat above using a different lot of primary standard solutions if available, to make 100 mg/L working control solutions of the same composition.

B. 10 mg/L Working Calibrator and Control

Group 1 - pipet 5 mL of 100 mg/L working calibrator into a 50 mL volumetric flask. Dilute to mark with methanol. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

Group 2 - pipet 5 mL of 100 mg/L working calibrator solution into a 50 mL volumetric flask. Dilute to mark with methanol. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

Group 3 - pipet 5 mL of 100 mg/L working calibrator solution into a 50 mL volumetric flask. Dilute to mark with methanol. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

Repeat above using working control solutions to make 10 mg/L control solutions of the same composition.

26. Internal Standards:

Primary Standards: Obtained in 1 mL ampoules from Cerilliant or equivalent.

Amphetamine-D ₁₁	1 mg/mL in methanol
Ephedrine-D ₃	1 mg/mL in methanol
Methamphetamine-D ₉	1 mg/mL in methanol
Methylenedioxyamphetamine (MDA)-D ₅	1 mg/mL in methanol
Methylenedioxyethylamphetamine (MDEA)-D ₅	1 mg/mL in methanol
Methylenemethamphetamine (MDMA)-D ₅	1 mg/mL in methanol

10 mg/L intermediate working internal standard pool.

Pipet 1.0 mL of 1.0 mg/mL from ampoules of Amphetamine-D₁₁, Methamphetamine-D₉, MDA-D₅, MDEA-D₅ and MDMA-D₅ to a 100 mL volumetric flask. Dilute to mark with methanol. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

20 mg/L intermediate Ephedrine-D₃ internal standard solution.

Pipet 1.0 from each of two Ephedrine-D₃ ampoules (2 mL) to a 100 mL volumetric flask. Dilute to mark with methanol. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

Working Internal Standard Solution.

Combined 100 mL of the 10 mg/L intermediate working internal standard pool with 100 mL of 20 mg/L intermediate Ephedrine-D₃ internal standard solution. The final concentration of Amphetamine-D₁₁, Methamphetamine-D₉, MDA-D₅, MDEA-D₅ and MDMA-D₅ in the working internal standard solution is 5 mg/L, and for Ephedrine-D₃ is 10 mg/L. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

27. Gas Chromatography Column:

Restek RTX-50 (Crossbond 100% methylphenyl polysiloxane). 30M x 0.25 mm ID x 0.25 µm DF, max temperature 320 °C. Model # 10523

EXTRACTION PROCEDURE

1. Obtain a list of amphetamine cases to be analyzed. (Refer to "Procedure for Work list Printing.") Review with supervisor for any updates of rush cases or other special instructions.
2. Find and collect all samples on the list and place in an empty rack.
3. Obtain enough 16 x 125 mm disposable culture tubes for calibrators, controls, and all requested cases. Label the tubes appropriately. Tubes should bear the entire toxicology number (e.g., YY-1234, not 1234), the specimen type and any dilution.
4. Pipet 1 mL of validated negative matrix or sample into 16 x 125 mm test tube labeled as to the contents.
5. Add appropriate amounts of working calibrator solutions for Group 1, 2 (and group 3 if needed) to negative matrix tubes as indicated below.

Four calibrators of Group 1 amphetamines, four calibrators of Group 2 amphetamines and, four calibrators of Group 3 (bath salts, if needed) and matrix-matched blank(s) should be run with each batch of samples. Positive calibrators are prepared at following concentrations:

0.1 mg/L - add 10 μ L of 10 mg/L working calibrator solution

0.5 mg/L - add 50 μ L of 10 mg/L working calibrator solution

1.0 mg/L - add 10 μ L of 100 mg/L working calibrator solution

1.5 mg/L - add 15 μ L of 100 mg/L working calibrator solution

Vortex 15 seconds to mix.

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

6. Spike the control tubes for each matrix for both Group 1 and Group 2 (and Group 3, if required):

0.5 mg/L - add 50 μ L of 10 mg/L working control solution

1.0 mg/L - add 10 μ L of 100 mg/L working control solution

Vortex 15 seconds to mix.

Note: These samples should be placed randomly in the batch.

7. Add 50 μ L of working internal standard pool (5 mg/L for all amphetamines, except for ephedrine at 10 mg/L) to all test tubes. The concentration of the internal standard in each sample is 0.25 mg/L except for Ephedrine-D₃, which is 0.5 mg/L.
8. Add 2 mL of 100 mM phosphate buffer (pH 6.0), mix & vortex 15 seconds to mix.
9. Sonicate sample for 20 minutes using an ultrasonic bath.
10. Centrifuge for 10 minutes at $\approx$ 3000 rpm.

11. Transfer the supernatant into the SPE Polycrom Clin II column and apply nitrogen at a pressure of 2-4 psi.

12. Wash column (All wash steps are pressurized at 2-4psi).

Pour 2 mL DI H₂O onto column
Pour 1 mL methanol onto column
Pour 1 mL ethyl acetate onto column
Dry column (2 minutes at 25 psi N₂)

13. Elute Drugs

Place labeled 10 mL conical centrifuge tubes under each column to collect eluate. Elute with 3 mL CH₂Cl₂ /IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute. Apply pressure as necessary to achieve the desired flow rate.

Note: Prepare elution solvent daily. Add IPA/NH₄OH, mix, then add CH₂Cl₂

14. Add 50 µL of 1% methanolic HCl. Evaporate to dryness at 40 °C.

Note: Samples should be removed as soon as possible after they are dry. Avoid overheating.

15. Add 200 µL of toluene, followed by 100 µL of TFAA to each tube. Immediately cap each tube after the addition of TFAA. Vortex 15 seconds to mix. and incubate tubes for 15 minutes at 70 °C in an incubation oven.

16. Remove tubes from the incubation oven and allow tubes to cool to room temperature. Add 2.0 mL of pH 9.8 buffer. Vortex 15 seconds to mix.

17. Centrifuge for ten minutes at ≈ 3000 RPM.

18. Using a pipet, transfer the upper toluene layer to a glass insert in an appropriately labeled vial, indicating aliquot and toxicology numbers (ex: 2-YY-xxxx), specimen type, dilution, analyst and date. 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.

19. Transfer the vials to the GCMS section for analysis. Also enter the date completed in the Dataease database, so the samples are not duplicated by another analyst.

CAUTION: DO NOT WAIT UNTIL THE FOLLOWING DAY TO DO THIS!

INSTRUMENTATION

Agilent MSD (6890 GC with 5973 Mass Spectrometer), Autosampler (Agilent 7683), and Agilent Chemstation with current revision of software. The method name for the amphetamine assay is MSnSIMAM.M, where n = instrument number. Each MSD used for this assay will have a method with this name in the method directory.

The following ions are monitored for each drug:

Amphetamine-D ₁₁ TFA	144, 128
Methamphetamine-D ₉ TFA	161, 123
MDA-D ₅ TFA	136, 280
MDMA-D ₅ TFA	158, 163
Ephedrine-D ₃ TFA	157, 113
MDEA-D ₅ TFA	173, 141
Amphetamine TFA	140, 118, 91
Methamphetamine TFA	154, 110, 118
MDA TFA	135, 162, 77
MDMA TFA	154, 162, 135
Ephedrine TFA	154, 110, 244
Pseudoephedrine TFA	154, 110, 69
Phentermine TFA	154, 91, 132
Phenylpropanolamine TFA	140, 69, 230
Dexfenfluramine TFA	168, 140, 159
MDEA TFA	168, 162, 140
PMA TFA	121, 148, 261

TOP LEVEL PARAMETERS

Method Information for amphetamines: C:\MSDCHEM\1\METHODS\MSnSIMAM.M (n = MS instrument)

Method Sections To Run:

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

Method Comments:

This is the amphetamine SIM method.

END OF TOP LEVEL PARAMETERS

INSTRUMENT CONTROL PARAMETERS

Sample Inlet	GC
Injection Source	GC ALS
Mass Spectrometer	Enabled

HP6890 GC METHOD

OVEN

Initial temp	110 °C (On)	Maximum temp	320 °C
Initial time	2.00 min	Equilibration time	0.50 min

<i>Ramps</i>			
#	Rate	Final temp	Final time
1	15.00	230	2.00
Post temp	290 °C		
Post time	10.00 min		
Run time	12.00 min		

<i>Front Inlet (Unknown)</i>		<i>Back Inlet ()</i>
Mode	Pulsed Splitless	
Initial temp	220 °C (On)	
Pressure	11.40 psi (On)	
Pulse pressure	30.0 psi	
Pulse time	0.80 min	
Purge flow	7.5 mL/min	
Purge time	0.70 min	
Total flow	10.3 mL/min	
Gas saver	On	
Saver flow	20.0 mL/min	
Saver time	3.00 min	
Gas type	Helium	

<i>Column 1</i>		<i>Column 2</i>
Capillary Column		(not installed)
Model Number:	Restek RTX50	
Max temperature	320 °C	
Nominal length	30.0 m	
Nominal diameter	250.00 µm	
Nominal film thickness	0.25 µm	
Mode	Constant flow	
Initial flow	1.0 mL/min	
Nominal init pressure	11.42 psi	
Average velocity	37 cm/sec	
Inlet	Front Inlet	
Outlet	MSD	
Outlet pressure	vacuum	

FRONT DETECTOR (NO DET)	BACK DETECTOR (NO DET)
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COLUMN COMP 1	COLUMN COMP 2
(No Detectors Installed)	(No Detectors Installed)

<i>Thermal Aux 2</i>	
Use: MSD Transfer Line Heater	
Description	
Initial temp	280 °C (On)
Initial time	0.00 min
# Rate Final temp Final time	
1 0.0(Off)	

<i>Post Run</i>	
Post Time	10.00 min
Oven Temperature	290 °C
Column 1 Flow	1.0 mL/min

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

MS ACQUISITION PARAMETERS

<i>General Information</i>	
Tune File	atune.u
Acquisition Mode	SIM
<i>MS Information</i>	
Solvent Delay	3.6 min
EM Absolute	False
EM Offset	200
Resulting EM Voltage	(varies)

[Sim Parameters]

<i>Group 1</i>			
Group ID	1		
Resolution	High		
Plot 1 Ion	69.1		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(69.1, 30)	(91.1, 30)	(110.1, 30)
	(113.1, 30)	(118.1, 30)	(123.1, 30)
	(128.1, 30)	(132.1, 30)	(140.1, 30)
	(144.1, 30)	(154.1, 30)	(157.1, 30)
	(159.1, 30)	(161.1, 30)	(168.1, 30)
	(203.1, 30)	(230.1, 30)	(244.1, 30)

<i>Group 2</i>			
Group ID	2		
Resolution	High		
Group Start Time	7.80		
Plot 1 Ion	77.1		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(77.1, 30)	(121.1, 30)	(135.1, 30)
	(136.1, 30)	(140.1, 30)	(141.1, 30)
	(148.1, 30)	(154.1, 30)	(158.1, 30)
	(162.1, 30)	(163.1, 30)	(168.1, 30)
	(173.1, 30)	(261.1, 30)	(280.1, 30)

[MSZones]

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

The method name for the bath salts is MSnBATHSALTQT.M where n= instrument number. .
Each MSD used for this assay will have a method with this name in the method directory.

Buphedrone TFA	168,105,140,110
Mephedrone TFA	119,154,110,273
Methylone TFA	121,154,110,150
MDPV	126,121,149,96

TOP LEVEL PARAMETERS

Method Information for bath salts: C:\MSDCHEM\1\METHODS\ MSnBATHSALTQT.M (n = MS instrument)

Method Sections To Run:

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

Method Comments:

This is the "Bath Salts" SIM method.

END OF TOP LEVEL PARAMETERS

INSTRUMENT CONTROL PARAMETERS

Sample Inlet	GC
Injection Source	GC ALS
Mass Spectrometer	Enabled

HP6890 GC METHOD

OVEN

Initial temp	110 °C (On)	Maximum temp	320 °C
Initial time	2.00 min	Equilibration time	0.50 min

<i>Ramps</i>			
#	Rate	Final temp	Final time
1	15.00	230	15.00
Post temp	290 °C		
Post time	10.00 min		
Run time	25.00 min		

<i>Front Inlet (Unknown)</i>		<i>Back Inlet ()</i>
Mode	Pulsed Splitless	
Initial temp	220 °C (On)	
Pressure	11.42 psi (On)	
Pulse pressure	30.0 psi	
Pulse time	0.80 min	
Purge flow	7.5 mL/min	
Purge time	0.70 min	
Total flow	10.3 mL/min	
Gas saver	On	
Saver flow	20.0 mL/min	
Saver time	3.00 min	

Gas type	Helium	
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<i>Column 1</i>		<i>Column 2</i>
Capillary Column		(not installed)
Model Number:	Restek RTX50	
Max temperature	320 °C	
Nominal length	30.0 m	
Nominal diameter	250.00 µm	
Nominal film thickness	0.25 µm	
Mode	Constant flow	
Initial flow	1.0 mL/min	
Nominal init pressure	11.42 psi	
Average velocity	37 cm/sec	
Inlet	Front Inlet	
Outlet	MSD	
Outlet pressure	vacuum	

<i>FRONT DETECTOR (NO DET)</i>	<i>BACK DETECTOR (NO DET)</i>
COLUMN COMP 1	COLUMN COMP 2
(No Detectors Installed)	(No Detectors Installed)

<i>Thermal Aux 2</i>	
Use: MSD Transfer Line Heater	
Description	
Initial temp	280 °C (On)
Initial time	0.00 min
# Rate Final temp Final time	
1 0.0(Off)	

<i>Post Run</i>	
Post Time	10.00 min
Oven Temperature	290 °C
Column 1 Flow	1.0 mL/min

<i>Injector</i>		
Time	Specifier	Parameter & Setpoint
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	
<i>Back Injector:</i>		
No parameters specified		

MS ACQUISITION PARAMETERS

<i>General Information</i>	
Tune File	atune.u
Acquisition Mode	SIM
<i>MS Information</i>	
Solvent Delay	4.6 min
EM Absolute	False
EM Offset	200
Resulting EM Voltage	(varies)

[Sim Parameters]

<i>Group 1</i>			
Group ID	1		
Resolution	High		
Plot 1 Ion	69.1		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(69.1, 30)	(91.1, 30)	(110.1, 30)
	(113.1, 30)	(118.1, 30)	(123.1, 30)
	(128.1, 30)	(132.1, 30)	(140.1, 30)
	(144.1, 30)	(154.1, 30)	(157.1, 30)
	(159.1, 30)	(161.1, 30)	(168.1, 30)
	(203.1, 30)	(230.1, 30)	(244.1, 30)

<i>Group 2</i>			
Group ID	2		
Resolution	High		
Group Start Time	7.20		
Plot 1 Ion	136.10		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(96.00, 30)	(105.00, 30)	(110.00, 30)
	(119.00, 30)	(121.00, 30)	(126.00, 30)
	(136.10, 30)	(140.00, 30)	(141.10, 30)
	(149.00, 30)	(150.00, 30)	(154.00, 30)
	(158.10, 30)	(163.10, 30)	(168.00, 30)
	(173.10, 30)	(273.00, 30)	(280.10, 30)

[MSZones]

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

END OF MS ACQUISITION PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

INSTRUMENT SETUP

A GCMS autotune must be performed at the start of each day of use.

All autosampler syringe wash vials are filled with methanol.

Prepare a sequence using the following steps.

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

1. On the Method and Run toolbar, under Sequence, select Load Sequence. Select default.s. Click on Select.
2. In the Method and Run toolbar, under Sequence, select Edit Sequence. At the top of the screen under Data Path, click on Browse. Under Select Data Path, click on the msdchem folder. The click 1 and then click on the Data folder to highlight it. In lower left of dialog box, select Make New Folder. A folder with the name New Folder is created under DATA. Right click on New Folder and Rename or double click to highlight the folder name and change the entry. Rename the file using the format 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 pre-loaded entries. Starting at the first empty field, enter sample or QC information. For samples, this would include aliquot number, laboratory number, specimen source, dilution if any (i.e., 2-11-2432 fem). If the sample has been diluted, enter the appropriate dilution factor in the Multiplier column. For QC samples use an appropriate designator. Enter the next sample in the batch in the next open field down the column.

4. In the Type column, select the corresponding sample type for each vial: Sample, Blank, Calibration or QC.
5. In the Vial column, click in the cell with the number 1, hold down the left mouse button and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column and Increment. Verify that the vial numbers are correct.
6. In the Method column, verify that the correct method is loaded in the first cell. Then click on the first cell containing the method name, hold down the left mouse and drag to the last vial number in the sequence (cells will be highlighted). Right click and select Fill Column, No Increment. Verify that the method for each vial is correct.
7. In the Data File column, in the cell corresponding to the first vial, enter the data file name in the format MSnMMDDx001, where n = instrument number, MM = month, DD = day, and x = a letter indicating the batch being run, i.e. MS20411a001. 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 Level column, verify that the correct level numbers are entered for calibrators in this batch.
10. Verify No Update is selected for all vials under Update Rf and Update Rt.
11. Review the information typed for the sequence. Correct any information as needed. Verify that the Data Path is C:\MSDCHEM\1\Data\current sequence name. Verify that the Method Path is C:\MSDCHEM\1\METHODS. Then Click ok.

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

Setting-Up a Subsequence

On the Sample Log Table:

1. Select "**Keyword**" for "Type"
2. Select "**DataPath**" for "Method/Keyword"
3. Under "Comment/KeywordString" type in the *new data path* for your subsequence ie: **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**).
4. The suffix of the data files must be different from that of the original; i.e.: MSNMMDDY001; **the subsequence data files must start with 1 again.**
5. After typing in the entire sequence, save sequence accordingly.

6. Go to Sequence → Simulate Sequence → Run Sequence.
7. A dialog box will pop-up: DataPath **C:\MSDCHEM\1\DATA\MSnMMDDYYy** does not exist. Edit Sample Log Table? → Click **No** if the sequence was set up correctly.
8. A 2nd dialog box will pop-up: Create **C:\MSDCHEM\1\DATA\MSnMMDDYYy**? → Click **Yes**.
9. A 3rd dialog box will pop-up: Sequence Verification Done! View it? → Click **Yes**.

Setting-Up a Subsequence with a Different Method

On the Sample Log Table:

1. After inserting the DataPath keywords and Commands, Insert a Row.
 2. Select “**Keyword**” for “Type”
 3. Select “**MethodPath**” for “Method/Keyword”
 4. Check that the method for each sample is changed to the new method.
 5. Follow Steps 5-9 in the “**Setting-Up a Subsequence**” Section (see previous section).
1. 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. Under Data File Directory verify that the data file path is C:\MSDCHEM\1\DATA\batch name.
 2. 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.
 3. 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 chain-of-custody verification of process steps to the printed sequence list.
 4. 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.
 5. 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.
 6. 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. Be sure to enter a copy of the sequence in the Sequence Logbook.

DATA TRANSFER AND PROCESSING

All processing and review are performed on a processing computer.

SAVE METHOD TO ECM

1. After the run finishes, the data files will be in the data subdirectory on the local chemstation and also automatically transferred to ECM. From the acquiring computer, make sure the proper method, the one used to acquire the data, is loaded. On the top toolbar under ECM click on Save Method to ECM.
2. Click on GCMS, the correct instrument name folder, the appropriate month and batch to which the method will be saved.

RETRIEVE BATCH FROM ECM

1. At the processing computer, click on Processing Data Analysis. Log on using your OCME network username and password.
2. On Enhanced Data Analysis screen, click on ECM at the top toolbar and select Retrieve entire sequence from ECM.
3. This will open up Openlab ECM screen. Select GCMS, then the appropriate instrument, the month, and, finally the batch to be retrieved. On the status line at the bottom of the screen that the batch is being retrieved. The batch will be downloaded to the following location:
C:\msdchem\1\ECM\Retrieve\batch name"

LOAD METHOD AND BATCH

1. On the left screen under the C drive, open C:\msdchem\1\ecm\retrieve
2. Under retrieve, click on the batch that was retrieved. When all files have been downloaded to the processing computer, verify that the appropriate method is present in the batch.
3. To load the method, right click on the method under the batch being processed and select load. This will bring up "Be sure changes are saved. Load now?" Click yes. If the method is not present, load the method by retrieving the method from ECM.
4. Click on any file in the batch to load it.

PERFORM BATCH CALIBRATION

Under enhanced data analysis:

1. Process the calibrators. Select Tools from the toolbar, DoLIST, and Quant, No Report (QT 1). Press Add, and OK. Select the files for this action to be performed on, in this case, calibrators only. Verify that the selected files are located in the correct subdirectory. Change the path if necessary. Click the → Arrow and Process.
2. Review the integrations of the targeted compounds for each calibrator, checking that the ion peaks are present and integrated correctly (i.e. the baseline is the most scientifically accurate one that can be drawn). Select View from the toolbar, QEDIT. Answer appropriately when prompted to save changes made to quantitation results when moving from file to file. Return to Data Analysis by selecting View from the toolbar, return to Data Analysis.
3. Update the existing calibration table (all levels). Select Calibrate, Update, Quick Levels Update. When prompted to clear responses, select YES. When asked to requant files before update, select NO. Select single data file/level option. Select the appropriate data file to associate with calibration level 1 (0.1 mg/L). Click OK. Repeat for remaining calibration levels (0.5, 1.0, 1.5 mg/L). Select level 3 when prompted to update retention times.
4. Load the file associated with level 3 (1.0 mg/L), by selecting File, Load Data File. Select Calibrate, Update One Level. Do NOT requant. Select Update One Level, select only Replace Qualifier Ion Relative Responses, and choose the corresponding existing level ID (#3). Click Do Update.
5. Review the Compound database. Double click on the internal standard listed on the left to reveal the compounds quantitated with it. Select the calibration tab to reveal compound responses, calibration curves, and r^2 . To disable a point on the calibration curve for a compound, delete its response from the table. Click OK or Cancel when review is complete.
6. Save Method before proceeding. Select Method from the toolbar, Save method, make sure that the path is correct. Save to OpenLab ECM at this time. On Update Calibration screen, select Update Level. Then click on Responses and Replace and on Retention Times and Replace. Then under Existing level ID, select the cal level to update and click on Do Update.
7. Requantitate the calibrators with the updated calibration curve. Select Tools from the toolbar, DoLIST, Requant, no report (QT 2), Add, and OK. Remove any existing commands. Select files to process. Click the → Arrow and Process. Review with QEDIT. Check the responses, retention times and ion ratios.
8. Regression correlation coefficient (r^2) for each analyte must be equal to or greater than 0.99.
9. Process controls and cases. Select Tools from the toolbar, DoLIST, Quant, No Report (QT 1), Add, and OK. Select appropriate files. Click the → Arrow and Process. Review with QEDIT. The blank must not contain detectable amounts of target analytes or significant interfering peaks.

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

BATCH CLEAN UP

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

ACCEPTANCE CRITERIA

1. Review the entire batch, checking that the ion peaks are present and integrated correctly (e.g., that the baseline is the most scientifically accurate one that can be drawn), that the ion ratios are $\pm 20\%$ of the average of a calibrator, that the peaks are $\pm 2\%$ of the calibrator retention times, and the peaks meet chromatography criteria. The blank must not contain detectable amounts of target analytes or significant interfering peaks. The blood controls must be within $\pm 20\%$ of the target value. For tissues, the controls are acceptable up to $\pm 30\%$.

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

2. Make copies of all controls, the r^2 report and the sequence list, enough to attach a set to each case in the batch.

REPORTING

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

1. Each case printout must have a copy of the sequence and all controls appended.

2. Concentrations greater than or equal to 0.1 mg/L are reported in mg/L. Results are truncated and reported to the hundredth of a mg/L (ex: 0.275 mg/L is reported as 0.27 mg/L).
3. Concentrations lower than 0.1 mg/L but meeting all other criteria are reported as “less than 0.1 mg/L”. If the drug is not detected, or the criteria are not met, the drug is reported as “not detected”.
4. Sample concentrations greater than the highest acceptable calibrator must be re-extracted with suitable dilution(s) to bring it within quantitative curve limits.
5. Review other findings in the case, especially GCMS and GC results to see if they are consistent with these GCMS findings. If there are discrepancies, schedule additional testing to resolve them. If in doubt, consult with a supervisor.
6. If the positive matrix controls are greater than $\pm 30\%$ of target for non-blood matrices, the samples may be reported qualitatively, as “detected” or “not detected”. If quantitative results are needed, the sample must be repeated.
7. If the concentration of ephedrine is less than 1.0 % of the pseudoephedrine concentration, or conversely, the concentration of pseudoephedrine is less than 1.0 % of the ephedrine concentration, the drug that is less than 1.0 % will be reported as “not detected”.

REFERENCES

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