

**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 par-methoxyamphetamine (PMA). It is also applied to the quantitation of “bath salts” including buphedrone, mephedrone, methylone, methylenedioxypropylone (MDPV), ethylone, n-ethylcathinone and 4-methylethcathinone.

Sympathomimetic amines and “bath salts” are extracted from biological specimens using solid phase extraction. The term “bath salts” refers to an emerging family of drugs containing one or more synthetic chemicals related to cathinone, an amphetamine-like stimulant found naturally in the khat plant. 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 incubated 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.

1. **Deionized water** (distilled can be substituted)
2. **Methanol** (Fisher Scientific - ACS Certified)
3. **Ammonium Hydroxide, NH<sub>4</sub>OH** (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. **Negative blood, serum, brain, liver**

Calf or sheep blood obtained from outside source or equivalent. Sodium fluoride is added as a preservative, and stored frozen (-10°C or lower). Human plasma/serum obtained from outside

source or equivalent, and stored frozen (-10°C or lower). Calf brain and liver obtained from outside source or equivalent. Homogenized and stored frozen (-10°C or lower). All matrices are validated as negative by in-house analysis.

**10. Polycrom Clin II Solid Phase Extraction Column. CEREX**

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

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

13. **Evaporation apparatus (e.g. Turbovap or SPEware)** connected to a nitrogen source.

14. **Sonicator**

15. **Vortex**

16. **Centrifuge**

17. **GCMS column, Restek RTX-50 30M x 0.25 mm ID x 0.25 µm DF, Model # 10523**

**18. 100 mM phosphate buffer (pH 6.0)**

Dissolve 3.4 g Na<sub>2</sub>HPO<sub>4</sub> and 24.2 g NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O in 1600 mL DI H<sub>2</sub>O.

Dilute to 2000 mL using DI H<sub>2</sub>O. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).

Store at room temperature in glass.

Stability: 1 month. Inspect each day before use for contamination.

19. **Eluting solvent: CH<sub>2</sub>Cl<sub>2</sub> /IPA/NH<sub>4</sub>OH (78/20/2).** Prepare fresh each day of use.

**20. 1% methanolic HCl.**

Add 50 mL of methanol to volumetric flask. Slowly add 1mL of concentrated hydrochloric acid into the flask and swirl flask slowly. Bring to the final volume of 100 mL with methanol.

21. **Trifluoroacetic anhydride (TFAA).** Aldrich Chemical.

22. **Toluene.** Fisher Scientific or equivalent or equivalent.

23. **Reference Standards:** Stock 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
Phenylpropanollamine	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
Methylenedioxypropylvalerone	1 mg/mL in methanol
Ethylone	1 mg/mL in methanol
n-ethylcathinone	1 mg/mL in methanol
4-methylethcathinone	1 mg/mL in methanol

## 24. Calibrators and controls

Calibrators and in-house controls are prepared according to the section "Preparation of GC Calibrators and Controls" with the following drugs in Groups 1-4.

<b>Group 1</b>	<b>Group 2</b>	<b>Group 3</b>	<b>Group 4</b>
Amphetamine	Ephedrine	Buphedrone	Ethylone
Methamphetamine	Fenfluramine	Mephedrone	n-ethylcathinone
MDA	MDEA	Methylone	4-methylethcathinone
MDMA	Phentermine	MDPV	
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 individual 1.0 mL 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 individual 1.0 mL 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 2 mL of 1 mg/mL stock solution (or 2 individual 1.0 mg/L ampoules) of buphedrone, mephedrone, methylone and MDPV to a 20 mL volumetric flask. Dilute to mark with methanol. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

Group 4 - pipet 2 mL of 1 mg/mL stock solution (or 2 individual 1.0 mg/L ampoules) of ethylone, n-ethylcathinone, 4-methylethcathinone to a 20 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 5mL 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 2 mL of 100 mg/L working calibrator solution into a 20 mL volumetric flask. Dilute to mark with methanol. Transfer into properly labeled container (include concentration, lot number, date prepared, initials of analyst).

Group 4 - pipet 2 mL of 100 mg/L working calibrator solution into a 20 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.

## 25. Internal Standards:

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

Amphetamine-D <sub>11</sub>	1 mg/mL in methanol
Ephedrine-D <sub>3</sub>	1 mg/mL in methanol
Methamphetamine-D <sub>9</sub>	1 mg/mL in methanol
Methylenedioxyamphetamine (MDA)-D <sub>5</sub>	1 mg/mL in methanol
Methylenedioxyethylamphetamine (MDEA)-D <sub>5</sub>	1 mg/mL in methanol
Methylenemethamphetamine (MDMA)-D <sub>5</sub>	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-D<sub>11</sub>, Methamphetamine-D<sub>9</sub>, MDA-D<sub>5</sub>, MDEA-D<sub>5</sub> and MDMA-D<sub>5</sub> 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-D<sub>3</sub> internal standard solution

Pipet 1.0 from each of two Ephedrine-D<sub>3</sub> 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-D<sub>3</sub> internal standard solution. The final concentration of Amphetamine-D<sub>11</sub>, Methamphetamine-D<sub>9</sub>, MDA-D<sub>5</sub>, MDEA-D<sub>5</sub> and MDMA-D<sub>5</sub> in the working internal standard solution is 5 mg/L, and for Ephedrine-D<sub>3</sub> is 10 mg/L. Transfer into

properly labeled container (include concentration, lot number, date prepared, initials of analyst.)

## EXTRACTION PROCEDURE

1. Obtain a list of amphetamine cases to be analyzed. 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. Aliquot 1 mL of validated negative matrix into each 16 x 25 mm tube labeled calibrator(s) or control(s). Aliquot 1 mL of sample into each appropriately labeled 16 x 125 mm tube
5. It should be noted that some specimens including but not limited to decompose blood, cavity blood or putrefied blood will need to be extracted neat and diluted 1:5. Prepare calibrators as follows using negative matrix:

Note: Calibrators are prepared separately for Groups 1 and 2, and 3-4. Group 1 amphetamines:

- 0.1 mg/L - add 10  $\mu$ L of 10 mg/L working Group 1 calibrator solution
- 0.5 mg/L - add 50  $\mu$ L of 10 mg/L working Group 1 calibrator solution
- 1.0 mg/L - add 10  $\mu$ L of 100 mg/L working Group 1 calibrator solution
- 1.5 mg/L - add 15  $\mu$ L of 100 mg/L working Group 1 calibrator solution

Vortex 15 seconds to mix.

Repeat for Group 2 amphetamines, using working Group 2 calibrator solution.

Repeat with Groups 3-4 bath salts, using working Groups 3 **and** 4 calibrator solutions, if needed.

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

6. Prepare controls as follows using negative matrix:

Note: Controls are prepared separately for Groups 1 and 2, and 3-4.

Group 1 amphetamines:

- 0.5 mg/L - add 50  $\mu$ L of 10 mg/L working Group 1 control solution
- 1.0 mg/L - add 10  $\mu$ L of 100 mg/L working Group 1 control solution

Vortex 15 seconds to mix.

Repeat for Group 2 amphetamines, using working Group 2 control solution.

*Repeat with Groups 3-4 bath salts, using working Groups 3 **and** 4 control solutions, if needed. **Note:** These samples should be placed randomly in the batch.*

7. A matrix matched blank must be included for each matrix type in the batch.
8. Add 50  $\mu\text{L}$  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- $\text{D}_3$ , which is 0.5 mg/L.
9. Add 2 mL of 100 mM phosphate buffer (pH 6.0). Vortex 15 seconds to mix.
10. Sonicate sample for 20 minutes using an ultrasonic bath.
11. Centrifuge for 10 minutes at  $\approx$  3000 rpm.
12. Decant the supernatant into the SPE Polycrom Clin II column and apply nitrogen at a pressure of 2-4 psi. If the sample does not flow evenly through the column, this step will necessitate additional phosphate buffer.
13. Wash column (All wash steps are pressurized at 2-4psi).
  - Pour 2 mL DI  $\text{H}_2\text{O}$  onto column
  - Pour 1 mL methanol onto column
  - Pour 1 mL ethyl acetate onto column
  - Dry column (2 minutes at 25 psi)
14. Prepare Elution Solvent
  - $\text{CH}_2\text{Cl}_2$  /IPA/ $\text{NH}_4\text{OH}$  (78/20/2) by mixing IPA/ $\text{NH}_4\text{OH}$ , followed by  $\text{CH}_2\text{Cl}_2$ .

**Note:** Prepare elution solvent daily.
15. Elute Drugs
  - Place labeled 10 mL conical centrifuge tubes under each column to collect eluate. Elute with 3 mL. Collect eluate at 1 to 2 mL/minute. Apply pressure as necessary to achieve the desired flow rate.
16. Add 50  $\mu\text{L}$  of 1% methanolic HCl. Evaporate to dryness at 40  $^\circ\text{C}$ .

**Note:** Samples should be removed as soon as possible after they are dry. Avoid overheating.
17. Add 200  $\mu\text{L}$  of toluene, followed by 100  $\mu\text{L}$  of TFAA to each tube. Immediately cap each tube after the addition of TFAA. Vortex 15 and incubate tubes for 15 minutes at 70  $^\circ\text{C}$  in an incubation oven.
18. Remove tubes from the incubation oven and allow tubes to cool to room temperature. Add 2.0 mL of pH 9.8 buffer and vortex for 5-10 seconds.
19. Centrifuge for 10 minutes at  $\approx$  3000 RPM.
20. 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. Physically check that the crimped seal is tight by attempting to rotate the seal. Crimp until tight, using a new seal if necessary. Samples may also be transferred into screw cap vials and capped immediately. Do not wait until all transfers have been made to seal the vials. Samples are ready for MS injection.

21. 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 <sub>11</sub> TFA	144, 128
Methamphetamine-D <sub>9</sub> TFA	161, 123
MDA-D <sub>5</sub> TFA	136, 280
MDMA-D <sub>5</sub> TFA	158, 163
Ephedrine-D <sub>3</sub> TFA	157, 113
MDEA-D <sub>5</sub> 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:

- Save Copy of Method With Data
- Pre-Run Cmd/Macro =
- 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	15.00
Post temp	290 °C		
Post time	10.00 min		
Run time	25.00 min		

<i>Front Inlet (Split/Splitless)</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	

<i>Column 1</i>		<i>Column 2</i>
Capillary Column		(not installed)
Model Number:	Restek RTX50 or equivalent	
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	38 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	
<b>Description</b>	
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>		
<b>Time</b>	<b>Specifier</b>	<b>Parameter &amp; Setpoint</b>
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)
		(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.

MDA-d <sub>5</sub> TFA	136, 280
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Buphedrone TFA	168,105,140,110
Mephedrone TFA	119,154,110,273
Methylone TFA	121,154,110,150
MDPV	126,121,149,96
Ethylone TFA	149,168,121, 150
n-ethylcathinone TFA	168,105,77
4-methylethcathinone TFA	119,168,140

### TOP LEVEL PARAMETERS

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

Method Sections To Run:

- Save Copy of Method With Data
- Pre-Run Cmd/Macro =
- 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		
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<i>Front Inlet (Split/Splitless)</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	

<i>Column 1</i>		<i>Column 2</i>
Capillary Column		(not installed)
Model Number:	Restek RTX-50	or equivalent
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	38 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	
<b>Description</b>	
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		
Group Start Time	7.00		
Plot 1 Ion	136.10		
Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(77.00, 30)	( 96.00, 30)	(105.00, 30)
	(110.00, 30)	(119.00, 30)	(121.00, 30)
	(126.00, 30)	(136.10, 30)	(140.00, 30)
	(149.00, 30)	(150.00, 30)	(154.00, 30)
	(168.00, 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**

An acceptable autotune must be obtained prior to batch analysis. Refer to the SOP "GCMS Tuning" for instructions.

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. MS3040315a. Click **OK** which will take you back to the sample log table.
3. In the Sample column verify the correctness of pre-filled entries. Starting at the first empty row, enter sample or QC information. For samples, this would include aliquot number, laboratory number, specimen source, and dilution if any (i.e., 2-15-1234 fem). 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..
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 including washes and reinjections

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 (e.g. lot numbers).
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\MSnMMDDYYy** where "n" is the instrument's number and "y" 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 2<sup>nd</sup> dialog box will pop-up: Create **C:\MSDCHEM\1\DATA\MSnMMDDYYy?** → Click **Yes**.
9. A 3<sup>rd</sup> 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).
12. 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.
  13. 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. MS2041215a) Select Save. The extension ".s" will automatically be added.
  14. Under **Sequence** select **Print Sequence**. Verify that **Brief Format** is selected and click on **OK**. The sequence will be printed. Apply the chain of custody labels to the printed sequence list.
  15. Use the printed sequence list to load vials into the appropriate autosampler positions. Check vial information against the sequence list and ensure that the vial is inserted in the correct numeric position in the autosampler. Date and initial the chain of custody label on the sequence printout, listing any comments, transfers, or exceptions.
  16. 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**.
  17. 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- EXPORTING RAW DATA FOR PROCESSING**

All processing and review of the processed data must be 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 - RAW DATA FOR PROCESSING**

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\MSnMMDDYx”.

### **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 Managers. 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.

### **LOAD METHOD AND BATCH –FIRST LEVEL REVIEW**

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. Verify that the appropriate method is present in the batch folder.
3. To load the method, right click on the method 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 – PROCESSING – FIRST LEVEL REVIEW

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. Regression correlation coefficient ( $r^2$ ) for each analyte must be equal to or greater than 0.99.
8. 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. Verify multipliers/dilution factors are applied.

9. 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.
10. 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.
11. 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.
12. Save files to ECM. Select ECM from the toolbar, select "Save multiple data files to ECM". Select all files.
13. 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. Acceptance range for calibrators is  $\pm 20\%$  of the target concentration. A calibrator may be dropped if outside of the acceptable range. However, the remaining acceptable calibrators must be re-processed and quantitative values for cases reported within the dynamic range of the acceptable calibration range.
2. Blood controls must pass integration, retention time ( $\pm 2\%$  of the calibrator retention times) , and peak shape criteria. Additionally, they must be within  $\pm 20\%$  of the target value. For tissues, the controls are acceptable up to  $\pm 30\%$  of the target value. The blank must not contain detectable amounts of target analytes or significant interfering peaks.
3. Ion ratios must be within  $\pm 20\%$  of the target ion ratio, as determined by the midpoint calibrator, for blood samples. For non-blood samples, the ion ratios may be accepted within  $\pm 30\%$ , if the analyte has met acceptance criteria in a blood matrix.
4. **Note:** Sometimes ion ratios will be out in exceptionally low or high concentrations, or in cases of coelutions. The analyst must evaluate this, and determine other appropriate actions as needed, such as dilution or reinjection (using methods described on pages 15-16).
5. For each analyte quantitative run the recovery/response of the internal standard(s) shall be monitored. 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  $\pm 30\%$  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. 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  $\pm 20\%$  of their average responses, the calculated amount of the spiked sample is  $\pm 20\%$  of the theoretical amount. If any of these conditions are not met than consult with a toxicology manager for further direction.

6. Make sufficient copies of the controls (in-house and external), calibration report, and the sequence list, enough to attach a set for each case in the batch.

## **SECOND LEVEL REVIEW**

The Second Level Reviewer will review the processed data in its entirety according to the acceptance criteria section of this SOP.

## **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. 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”.

8. Submit the case file with all reports for Third Level Review.

### **THIRD LEVEL REVIEW (FINAL REVIEW)**

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

### **REFERENCES**

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4. Agilent 6890 GC System Standard Operating Procedures.
5. Agilent 5973 & 5973 Network Mass Selective Detector Installation Guide.
6. Agilent 5973 & 5973 Network Mass Selective Detector Users Guide.
7. Agilent 5973 & 5973 Network Mass Selective Detector Standard Operating Procedures.
8. SPEware Corp Cerex Applications Manual.
9. System 48 Processor Users Guide.
10. Turbovap Users Guide.

## REVISION HISTORY

Ver 03.08.2013	1. Revision history implemented.
Ver 04.20.2015	1. Addition of ethylone, n-ethylcathinone and 4-methylethcathinone to the bath salts panel.
Ver 08.31.2015	1. Added internal standard acceptance criteria 2. Defined the level of review(s)

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