

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

**SYMPATHOMIMETIC AMINES
by
SOLID PHASE EXTRACTION
and
GAS CHROMATOGRAPHY/MASS SPECTROMETRY
(FULL SCAN)**

PRINCIPLE

This method is applied to qualitative analysis 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). This method also applies to the qualitative analysis of "bath salts" including buphedrone, mephedrone, methylone and methylenedioxypropylone (MDPV).

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.

This is a qualitative procedure using a single calibrator of 1.0 mg/L. Controls for the above listed amphetamines and any additional amphetamine class compounds are prepared with each batch.

Samples are analyzed by GCMS in the full scan mode to determine the presence of an amphetamine class compound. Quantitation of positive samples is performed using a fresh aliquot. Quantitative analysis is performed by GCMS using SIM mode with deuterated internal standards where available.

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:

Uncontrolled Copy

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. Reagents from a different source are acceptable if equivalent.

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 (IPA).** Fisher Scientific or equivalent.

7. **Sodium Phosphate Monobasic** Certified ACS Fisher Scientific or equivalent.
8. **Sodium Phosphate Dibasic** Certified ACS 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 Na₂HPO₄ and 24.28 g NaH₂PO₄ H₂O in 1000 mL DI H₂O.
 Dilute to 2000 mL using DI H₂O. Mix. Adjust pH to 6.0 ± 0.1 with monobasic sodium phosphate (lowers pH) or dibasic sodium phosphate (raises pH).

 Storage: in glass about 4 °C in glass at refrigerator.

 Stability: 1 month; inspect each day of use for contamination.
20. **Eluting solvent: CH₂Cl₂ /IPA/NH₄OH (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. **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
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" with the following drugs used in for Groups 1-3.

<i>Group 1</i>	<i>Group 2</i>	<i>Group 3</i>
Amphetamine	Ephedrine	Buphedrone
Methamphetamine	Fenfluramine	Mephedrone
MDA	MDEA	Methylone
MDMA	Phentermine	Methylenedioxypropylamphetamine
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 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 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:

50 mg/L methapyrilene internal standard.

See SOP section "Calibrators and Controls for Basic Drugs by GC and GCMS" for preparation.

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. 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 specimen or sample into 16 x 125 mm test tube labeled as to the contents.
5. The calibrator 1.0 mg/L is spiked with 10 µL of 100 mg/L calibrator solution (group 1, 2 and 3 are separate tubes).
6. Spike the 0.2 mg/L control tubes (group 1, 2 and 3 are separate tubes) with control solutions (20 µL of 10 mg/L control solution). Vortex 15 seconds to mix. Place this control randomly within the batch.
7. Add 20 µL of 50 mg/L methapyrilene internal standard to each tube. Vortex 15 seconds to mix. The concentration of internal standard in each sample is 1.0 mg/L.
8. Add 2 mL of 100 mM phosphate buffer (pH 6.0), Vortex 15 seconds to mix.
9. Sonicate sample for 20 minutes using an ultrasonic bath.
10. Centrifuge for 10 minutes at ≈ 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 2-4 psi).

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)

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 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 and vortex for 5 to 10 seconds.

17. Centrifuge for 10 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-xxx), 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.

IMPORTANT: 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 this assay is MSNAMP.M, where N = instrument number. Each MSD used for sympathomimetic amines analysis will have a method with this name in the method directory.

TOP LEVEL PARAMETERS

Method Information For: C:\MSDCHEM\1\METHODS\MSnAMP.M (n = instrument number)

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 and bath salts SCAN 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	0.5 min

<i>Ramps</i>			
#	Rate	Final temp	Final time
1	15.00	230	8.00
2	0.0(Off)		
Post temp	290 °C		
Post time	5.00 min		
Run time	18.00 min		

<i>Front Inlet (Unknown)</i>		<i>Back Inlet ()</i>
Mode	Pulsed Splitless	
Initial temp	220 °C (On)	
Pressure	11.23 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	11.23 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	
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	5.00 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	
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	Scan		
<i>MS Information</i>			
Solvent Delay	4.00 min		
(Scan Parameters)			
Low Mass	35.0		
High mass	300.0		
Threshold	150		
Sample #	2	A/D Samples	4
Plot 2 low mass	283.3		
Plot 2 high mass	284.3		

[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

The GCMS must be autotuned 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 relog in. 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. 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 **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 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**).
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).

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** (e.g., MPM). 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. MS2041211a) 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 preprinted labels for documenting verification of process steps to the printed sequence list.
15. 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.
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

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 the 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 calibrator. 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, calibrator 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 the 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. 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

4. 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 . Click OK or Cancel when review is complete
5. Save Method before proceeding. Select Method from the toolbar, Save method, make sure that the path is correct. Save to ECM can be cancelled at this point, as the method will be saved to ECM after printing.
6. Requantitate the calibrator 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.
7. 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.
8. Review all controls and case samples with QEDIT. The blank must not contain detectable amounts of target analytes or significant interfering peaks.
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. 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. Internal controls must be analyzed in accordance with the standard laboratory procedures to determine that the drugs can be detected. All drugs in the controls must be detected.

2. The negative control (blank) must not contain any of the target analytes.

SECOND LEVEL REVIEW

1. Check chromatography of all injections. Examine the peak shape and note if any peaks show non-Gaussian shape. Identify unresolved peaks and peaks with shoulders on either side. Address any noted problems before proceeding with data review. Consult a supervisor about any unusual events.
2. Check that all significant peaks in the chromatogram are integrated. If the printout allows, check if the baseline used to integrate is appropriate. The analyst should consult with a supervisor about any unusual events, such as the presence of overload peaks or poor recovery.
3. Check that all components of the calibrator and controls are present and that each peak is properly assigned. Correct any errors. If any calibration compounds do not extract, or if unexpected multiple peaks are detected for a calibration compound, the analyst must consult with a supervisor.
4. Check the blank for significant peaks that might co-elute with a compound of interest. There is some room for judgment in this step, but the analyst should consult with a supervisor if there is ANY question.
5. Review QC samples, determine if the controls meet all acceptance criteria.

REPORTING

1. After the batch has been reviewed and printed, it must be reported, using the following guidelines:
2. Each case printout must have a copy of the sequence and all controls appended.
3. Review the printouts, and report the results as "(drug X) detected", or "sympathomimetic amines not detected".
4. If an amphetamine class drug or bath salt is detected, schedule quantitative analysis for the specific drug.
5. 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

1. Clark's Identification and Isolation of Drugs in Pharmaceuticals, Body Fluids and Post-Mortem Materials, Ed. A.C. Moffat, The Pharmaceutical Press, London, Second Edition, 1986.
2. Agilent 6890 GC System Installation Guide.
3. Agilent 6890 GC System Users Guide.
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.

Uncontrolled Copy

Uncontrolled Copy