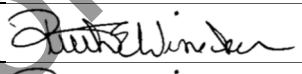
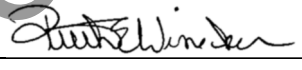
SOP-060 Chromatography, Tune Evaluation and Troubleshooting

Table of Contents

SOP Name: Review of Evidence Storage Room Access (Room 2607)		SOP #: 060
North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	Revision:	Revision Date/Initials:
Approving Authority Name	Approving Authority Signature	Approval Date
Ruth E. Winecker, Ph.D.		04/16/2015
Ruth E. Winecker, Ph.D.		06/10/2016
Ruth E. Winecker, Ph.D.		12/06/2017

1. **Principle**

1.1. The Toxicology Laboratory utilizes multiple instruments, in multiple configurations to carry out daily operations. This procedure outlines the steps taken to evaluate the performance of the laboratory's Gas Chromatography/Mass spectrometers (GC/MS), GC-NPD (Nitrogen Phosphorus Detectors), and GC-FID (Flame Ionization Detectors) prior to analyzing casework.

1.2. Chromatography on the various instruments is evaluated though the use of a test-mix which is comprised of commonly encountered drugs and compounds which are susceptible to poor peak shape when the instrument has active sites or dirty injection port liners.

1.3. All quadrupole mass spectrometers are tuned with a compound of known mass spectrum (PFTBA - perfluorotributylamine). All instruments are tuned prior to use each day loads are initiated. Tuning allows mass spectral libraries to be used for searching unknown peaks and confirming known peaks. The tuning program uses three ions from the PFTBA spectrum for its tuning: m/z 69, 219 and 502. Further, the abundance of the C13 isotopes at 70, 220, and 503 serve as a check of tuning.

2. **Mass Spectrometer Evaluation**

2.1. This section is designed to provide the parameters used for evaluation of the tune and test mix (see Standard logbook for test mix concentrations and components) to determine if the GC/MS is ready for an analytical run. The Test mix evaluation is broken into 2 parts: GC evaluation (separation and chromatography) and MS evaluation (spectrum evaluation).

2.2. Mass Spectrometer Agilent

2.2.1. Load the method "splitob" and allow the instrument to complete equilibration. Initiate a tune by clicking on the "instrument" tab from the instrument control view. From the drop down menu select "Tune MSD…" A dialog box labeled "Select Tune Type" will appear. Chose Tune MSD and hit OK. The tune associated with that method will begin.

2.2.2. Tune Evaluation

2.2.2.1. Peak width: Look at the Pw50 value for the 69/219/502 ions. These Pw50 values should be 0.50 ±0.10.

2.2.2.2. Inspect the mass peaks in the upper profile part of the display for good peak shape (no peak splitting and resolution between mass 502 and 503)

2.2.2.3. The 69 ion abundance should be greater than 200,000 counts.

2.2.2.4. The 502 ion should have a relative abundance of > 3%.

2.2.2.5. Isotope ratios should be ± 20 % of target values (1.08 ± 0.216 for m/z 70, 4.32 ± 0.864 for m/z 220 and 10.09 ± 2.01 for m/z 503).

2.2.2.6. The 18 and the 28 ions should be less than 10% relative to the 69 ion.

2.3. Mass Spectrometer Thermo (DSQ 1)

2.3.1. Autotune Evaluation

2.3.1.1. Peak width: Look at the Pw50 value for the 69/219/502 ions These Pw50 values should be 0.55 ±0.15.

2.3.1.2. The 69 ion abundance should be greater than 900,000 counts.

2.3.1.3. The Leak Check should be less than 3% of reference.

2.3.1.4. The 18 and the 28 ions should be less than 15% relative to the 69 ion.

2.4. Mass Spectrometer Thermo (DSQ 2)

2.4.1. Load the "ANFID" method and allow the instrument to equilibrate. To initiate a tune click on the tune icon on the desktop. Click "tune" on the menu bar. In the drop down menu click "Automatic Tune". The "Automatic Tune" dialogue box will appear. Click the maintenance tune option.

2.4.2. Autotune Evaluation

2.4.2.1. Peak width: Look at the Pw50 value for the 69/219/502 ions. These Pw50 values should be 0.55 ±0.15.

2.4.2.2. The 69 ion abundance should be greater than 9,000,000 counts.

2.4.2.3. The Leak Check should be less than 3% of reference.

2.4.2.4. The 18 and the 28 ions should be less than 15% relative to the 69 ion.

2.5. GC Column Test mix Evaluation

2.5.1. Make sure that the MS is on and collecting data early enough to show the

first analyte in the test mix, amphetamine.

2.5.2. Look for all analytes contained in the test mix.

2.5.3. Make sure that peaks are Gaussian shaped. If tailing is suspected after visual inspection calculate the asymmetry of Threo Bupropion (A= (CB/AC at 10% of the peak height). A value of 1.5 or lower is acceptable.

2.5.4. The ratio of Norfluoxetine/Fluoxetine should be at least 1/3.

2.5.5. Sertraline and Norsertraline should not co-elute >15%.

2.5.6. Make sure that Oxycodone is detected in the chromatogram (may appear as a trailing shoulder on the Nordiazepam peak).

2.5.7. Make sure the Alphaprodine peak abundance is greater than 2,000,000 counts

3. **GC NPD Evaluation**

3.1. This section is designed to provide the parameters used for evaluation of the test mix to determine if the GC/NPD is ready for an analytical run. The test mix evaluation is broken into 2 parts: GC evaluation (separation and chromatography) and NPD evaluation.

3.2. Test mix Evaluation

3.2.1. Make sure that the instrument saves data one minute prior to the first eluting analyte, Amphetamine, and two minutes after the last analyte, Trazodone.

3.2.2. Ensure that each analyte in the test mix is present in the chromatogram.

3.2.3. Make sure that peaks are Gaussian shaped. If tailing is suspected after visual inspection calculate the asymmetry of Threo Bupropion (A= (CB/AC at 10% of the peak height). A value of 1.5 or lower is acceptable.

3.2.4. Make sure that Mirtazapine and Cyclobenzaprine have baseline separation.

3.2.5. Sertraline and Norsertraline should not co-elute greater than15% above the baseline.

3.2.6. Ensure that the Alphaprodine peak exhibits a peak area of at least 1.5 million counts.

3.3. NPD Evaluation

3.3.1. Inspect the beginning of the chromatogram for a dropping slope that includes amphetamine. If this slope is present, bring it to the attention of the GC instrument chemist and stop the analytical run.

3.3.2. Inspect the baseline. If the baseline rises more than 15 pA from the beginning of the chromatogram to the end of the chromatogram bring it to the attention of the GC instrument chemist and stop the analytical run.

4. **Acid Neutral test injection evaluation (GC/MS/FID).**

4.1. Prepare an autosampler vial (with insert) made of a 50/50 mix of Acid/Neutral standard 1 and mephobarbital internal standard. Inject it on the GC/MS/FID instrument set up to run the acid/neutral method "ANFID".

4.2. Evaluate the peak heights of the internal standard in relation to Metaxalone, Primidone, Phenytoin, Phenobarbital and Lamotrigine.

4.2.1. Mephobarbital, Primidone, Phenytoin, and Phenobarbital should be approximately the same peak height.

4.2.2. Metaxalone should be 50% of the peak height of the ISTD.

4.2.3. Lamotrigine should be 75% of the peak height of the ISTD.

4.3. Make sure that the peaks are Gaussian shaped. If tailing is suspected after visual inspection calculate the asymmetry of Phenobarbital (A= (CB/AC at 10% of the peak height). Values of 1.5 or lower are acceptable.

5. **Discrepancies**

5.1. If any of these criteria are not met, see the troubleshooting section and/or bring the data to the attention of the instrument chemist.

6. **Troubleshooting-** general troubleshooting guides are available in the gclc and gcms folders of the instrument data folder on the share drive (S:).

6.1. No peaks:

6.1.1. Incorrect sample concentration,
6.1.2. No analytes present (poor recovery),
6.1.3. Syringe missing or not installed correctly or needle bent,
6.1.4. Empty sample vial,
6.1.5. Injection in split mode instead of splitless mode

6.2. Tailing Peaks:
- 6.2.1. Active sites in sample path
- 6.2.2. Injection too large
- 6.2.3. Injection port too cool
- 6.2.4. Column flow too low
- 6.2.5. GC/MSD interface or ion source too cool

6.3. Rising Baseline:
- 6.3.1. Column bleed
- 6.3.2. Other contamination

6.4. Retention Time Drift:
- 6.4.1. Septum has a hole
- 6.4.2. Column has been shortened (shorter RT)
- 6.4.3. Old column (shorter RT)
- 6.4.4. Active sites in sample path (longer RT)
- 6.4.5. Reduced column flow (longer RT)
- 6.4.6. Injection port leak (longer RT)
- 6.4.7. Initial oven temperature changed (up = shorter RT, down = longer RT)

6.5. Poor Sensitivity
- 6.5.1. Poor Extraction Recovery
- 6.5.2. Incorrect tuning
- 6.5.3. Tune file does not match type of analysis
- 6.5.4. Incorrect temperatures
- 6.5.5. Incorrect sample concentration
- 6.5.6. Leaking injection port
- 6.5.7. Incorrect split ratio
- 6.5.8. Purge off time in splitless mode too short
- 6.5.9. Dirty ion source
- 6.5.10. Air leak
- 6.5.11. Poor filament operation

7. **References**

7.1. Agilent 5973/5975 MSD Reference Collection

7.2. Thermo DSQ I/II User Guides

7.3. Semi-volatile Analysis Using an Inertness Performance Tested Agilent J&W DB-5ms Ultra Inert Column. Agilent Application Note. This reference is provided as an example of how to properly use a test mix for evaluation of chromatography.

- 7.3.1. http://www.crawfordscientific.com/downloads/pdf_new/GC/Semivolatile_Analysis_Using_DB-5msUI_5989-8616EN.pdf

7.4. Thermo Scientific Trace GC Ultra Maintenance and Troubleshooting Manual

7.4.1. http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/cmd-support/trace-gc-ultra/operations-and-maintenance/maintenance-instructions/Maintenance-Troubleshooting-Manual-March-2011.pdf