Chapter 11: Gas Chromatography/Mass Spectrometry (GC-MS)

1. Purpose / Scope

This procedure provides direction for the initial setup, performance checks and usage of gas chromatograph – mass spectrometer instruments in the Drug Chemistry Unit of the Wake County Bureau of Forensic Services.

2. Definitions

Performance verification - The initial confirmation of the reliability of a previously or externally validated method or instrument.

Quality control check - Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.

Reference Material - Material sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

3. Equipment, Materials and Reagents

3.1. Equipment

3.1.1. Agilent 8890 Gas Chromatograph with Agilent 5977 Series Mass Selective Detector with Agilent Automatic Liquid Sampler and tray

3.1.2. Computer with Agilent MassHunter Workstation GC/MS Data Acquisition Software and Analytical MSD Productivity ChemStation Software

3.2. Reference Materials

3.2.1. Multi-component drug solution containing alprazolam, clonazepam, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam and temazepam

3.3. Materials

3.3.1. Sample vials, caps and inserts

3.3.2. ALS Syringe, 10µl straight, fixed needle, 23/42/HP

3.3.3. DB5-MS Column, 30 m X 0.250 mm X 0.25 µm

3.3.4. Agilent inlet liner, split, single taper with glass wool, deactivated

3.3.5. Merlin microseal

3.3.6. Non-stick septa, 11mm

3.3.7. Septum wrench

3.3.8. Tweezers

3.3.9. Clean, lint free, non-nylon gloves

3.3.10. Wrenches, ¼ inch and ½ inch

3.3.11. Gold plated inlet seal with cross and 0.375 outer diameter washer

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3.3.12. Star or Torx screwdriver
3.3.13. Flat head screwdriver, large
3.3.14. Hex key, 5 mm
3.3.15. Hex ball driver, 1.5 mm
3.3.16. Hex ball driver, 2.0 mm
3.3.17. Wrench, open-end, 10 mm
3.3.18. Alumina abrasive powder
3.3.19. Cotton swabs
3.3.20. Ultrasonic bath

3.4. Commercial Reagents

3.4.1. Methanol, Optima or GC Resolv grade
3.4.2. Hexanes, Optima grade
3.4.3. Chloroform, Optima grade
3.4.4. Acetonitrile, Optima grade
3.4.5. Ethyl acetate, Optima grade
3.4.6. Methylene chloride, Optima or Pesticide grade
3.4.7. Helium gas, Grade 5.0
3.4.8. Perfluorotributylamine [PFTBA], neat

4. Standards and Controls

4.1. An electronic GC-MS logbook shall be maintained. The logbook shall contain the GC-MS Activity Log, GC-MS Daily QCC Log, the GC-MS Monthly QCC Log, the GC-MS Maintenance Log and any manufacturer's certificates, monthly and daily QCC's, sequence logs and maintenance documentation.

4.1.1. The logbook shall contain the Drug Chemistry GC-MS activity log.

4.1.1.1. Record the date, data file name, initials of operator, GC-MS method used, NQCC results, and any additional comments (if applicable) for each sample analyzed on the Drug Chemistry GC-MS Activity Log.

4.1.1.1.1. If samples are rerun for any reason, record a new entry on the Drug Chemistry GC-MS Activity Log.

4.1.1.2. Record any error messages on the Drug Chemistry GC-MS Activity Log.

4.1.2. The logbook shall contain the Drug Chemistry GC-MS daily QCC log.

4.1.2.1. Record all Daily QCC's on the Daily QCC log with the date, time, initials, and any comments.

4.1.2.2. Save Daily QCC's in the instrument logbook folder.

4.1.3. The logbook shall contain the monthly QCC data. Other reference material retention time data may be maintained in the logbook.

4.1.4. The logbook shall contain the GC-MS maintenance log.

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4.2. When the GC-MS has been placed out of service for maintenance, malfunction or leaving direct control of the Laboratory, the Drug Chemistry Technical Leader shall evaluate the instrument and determine if any additional quality control checks are needed to ensure instrument performance. At a minimum, a Daily QCC must be successfully performed prior to placing the instrument back in service.

4.2.1. If maintenance is performed that may affect retention times, a monthly QCC on all approved methods shall be performed before the instrument is placed back in service.

4.3. The Drug Chemist shall record any malfunctions or error messages in the GC-MS Activity Log, notify the Drug Chemistry Technical Leader of any malfunctions or error messages and place the instrument out of service by marking the GC-MS Activity Log "Out of Service."

4.4. The Drug Chemistry Technical Leader shall correct any problems with the instrument or request service. The Drug Chemistry Technical Leader shall examine the effect(s), if any, of a malfunction or error message on analysis results and implement the Wake County Bureau of Forensic Services Laboratory Administrative Procedure for Corrective and Preventive Action as required.

4.5. Negative Quality Control Check

4.5.1. Negative QCC's are performed prior to each sample injection.

4.6. Daily Quality Control Check – Autotune

4.6.1. Perform an Autotune (atune) with Perfluorotributylamine (PFTBA) as the tuning standard prior to beginning the first sample sequence each day the instrument is in use.

4.6.1.1. Sample sequences that continue overnight may be allowed to complete without performing a new tune provided that they do not extend more than thirty-six hours beyond the time of the tune.

4.6.2. Compare the atune report to previous ones. Record any major variations on the Daily QCC log and notify the Drug Chemistry Technical Leader.

4.6.3. The mass assignments of the tuning masses, 69.00, 219.00, and 502.00, in the upper part of the tune report must be within +/- 0.2 amu.

4.6.4. The peak widths on the tune report for masses 69.00, 219.00, and 502.00, must be within +/- 0.10 amu of 0.55 (i.e., 0.45-0.65) amu at 50% peak height (PW50) and the peaks should generally be smooth and symmetrical.

4.6.5. The base peak on the tune report must be identified as mass 69 or 219. The relative abundance ratio of mass 219 to mass 69 must be > 40% and the relative abundance ratio of mass 502 to mass 69 must be > 2.4%.

4.6.6. The 70/69 isotopic ratio must be from 0.5 – 1.6, the 220/219 ratio must be from 3.2 – 5.4, and the 503/502 the ratio must be from 7.9 – 12.3.

4.6.7. The abundance of any peaks less than 69 amu should not be greater than 10 % of the abundance of the base peak.

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4.6.7.1. Peaks at 18, 28 or 32 amu are indicative of water, nitrogen and oxygen, respectively, and may indicate an air leak. Other peaks may indicate gas impurities.

4.6.7.2. If an air leak is detected, isolate the leak and tighten fittings to correct the leak and perform another atune.

4.6.7.3. If the problem persists, place the instrument out of service by marking the activity log "out of service" and notify the Drug Chemistry Technical Leader.

4.6.7.4. The Drug Chemistry Technical Leader shall correct any problems with the instrument or request service.

4.6.8. If any parameter does not meet the requirements, document the deviation on the GC-MS daily QCC log.

4.6.8.1. Perform another atune.

4.6.8.2. If the problem persists, place the instrument out of service by marking the activity log "out of service" and notify the Drug Chemistry Technical Leader.

4.6.8.3. The Drug Chemistry Technical Leader shall correct any problems with the instrument or request service.

4.6.8.4. The daily QCC must be successfully completed prior to placing the instrument back in service.

4.6.9. Save the atune report in the instrument logbook folder and record the results in the GC-MS daily QCC log.

4.7. Annual/Monthly Quality Control Check

4.7.1. The multi-component drug solution shall be injected on all approved methods annually to verify instrument performance.

4.7.2. The multi-component drug solution shall be injected on all approved methods after source maintenance, column maintenance, or gold seal maintenance to verify instrument performance.

4.7.3. The multi-component drug solution shall be injected on the Low3, Screen2, and Cannabinoid2 method each month the instrument is in use.

4.7.3.1. The solution shall be run during the first seven calendar days of each month.

4.7.3.1.1. If the standard solution is not run during the first seven calendar days of the month, the instrument shall be out of service until the standard solution is successfully run.

4.7.4. If any other approved method (ISO3, Slow2, etc) is needed, the multi-component drug solution shall be injected on the applicable instrument and method prior to use for casework. The use of this method shall be considered valid for 30 days.

4.7.5. Name the multi-component reference material solution data files with "MC" followed by the numerical year and month designation and the method name. Name

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the corresponding solvent blank with the same designation followed by "-b", indicating that it is a blank.

Example: The name of a standard solution run in January, 2099 on the SCREEN method would be "MC209901SCREEN" and the blank would be "MC209901SCREEN-b."

4.7.6. Visually examine the TIC of the monthly QCC solution for chromatographic quality and resolution. All components must exhibit visually symmetrical peaks that are visually baseline resolved or for peaks separated by 0.2 minutes or less, visually resolved at half height.

4.7.7. Perform a GC retention time comparison for the Oxazepam, Temazepam and Alprazolam components of the monthly QCC solution to those of the previous monthly QCC solution run.

4.7.8. Perform a mass spectral comparison for the Oxazepam, Temazepam and Alprazolam components of the monthly QCC solution to reference material.

4.7.8.1. Record any component that does not have a positive comparison on the instrument monthly QCC log, place the instrument out of service by marking the GC-MS activity log "out of service" and notify the Drug Chemistry Technical Leader.

4.7.8.2. The Drug Chemistry Technical Leader shall correct any problems with the instrument or request service. The Drug Chemistry Technical Leader shall examine the effect(s), if any, on analysis results and implement the Wake County Bureau of Forensic Services Laboratory Administrative Procedure for Corrective and Preventive Action as required.

4.7.8.3. The monthly QCC must be successfully completed prior to placing the instrument back in service.

4.7.9. Save the annual/monthly QCC TIC with the retention times displayed, the mass spectrum of Oxazepam, Temazepam and Alprazolam and the corresponding blank TIC.

4.7.9.1. Record the lot number of the standard solution on the TIC.

4.7.9.2. Save the generated data in the instrument logbook folder.

4.7.9.3. Record the annual/monthly check in the monthly QCC log.

4.7.10. Additional reference material solutions may be run on a monthly basis to establish retention times.

4.8. Performance Verification for New Instrumentation

4.8.1. New GC-MS instruments shall be installed by a manufacturer representative or approved vendor according to the manufacturer's guidelines.

4.8.2. Prior to use, perform daily QCC's on three separate days. The daily QCC's must meet all specified requirements.

4.8.3. Prior to use, analyze the multi-component drug solution on each method on three separate days.

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4.8.3.1. The mass spectra of each component must have a positive mass spectral comparison to reference material.

4.8.3.2. The retention times of each component must have a positive GC retention time comparison to reference material.

4.8.4. Label the instrument data with the lot number of the reference material and date. Record the performance verification in the GC-MS logbook.

4.8.5. The performance verification must be reviewed and approved by the Drug Chemistry Technical Leader prior to the instrument being used for casework. The Drug Chemistry Technical Leader shall record the review and approval in the GC-MS logbook.

5. Maintenance

5.1. Place the instrument out of service prior to performing any column or mass spectrometer maintenance by marking the GC-MS activity log "Out of Service." When the instrument is ready to be returned to service, mark the GC-MS activity log "Back in Service."

5.2. Record all maintenance on the GC-MS maintenance log at the time it is performed with the name of the person performing the maintenance or repairs, the date, the time, a description of the maintenance or repairs and a list of any parts replaced, the type of post maintenance QCC performed, and any additional comments.

5.3. Record the length of column trimmed in the maintenance log. If the column is trimmed, the instrument shall be out of service until a monthly QCC on all approved methods is successfully completed.

5.3.1. Reference materials ran prior to the column maintenance shall not be used for retention time comparison after the column maintenance.

5.3.2. The Drug Chemistry Technical Leader shall update the instrument log when the instrument is ready to be used for casework and save any generated data in the instrument logbook folder.

5.4. Suggested Routine maintenance – The routine maintenance schedule is a suggested guideline. The maintenance schedule will be determined by the Drug Chemistry Technical Leader based upon instrument usage and performance.

5.4.1. Wash Vials

5.4.1.1. Rinse monthly and fill as needed when in use. Use methanol in the first wash vial and ethyl acetate in the second wash vial.

5.4.1.2. Required post-maintenance check: None.

5.4.2. Septum or Merlin microseal

5.4.2.1. For septum, replace weekly, at a minimum, when in use.

5.4.2.2. For Merlin microseal, replace yearly at a minimum when in use.

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5.4.2.2.1. Select [Maintenance], [Perform Maintenance], [Replace Septum], [Start Maintenance].
5.4.2.2.2. Be careful - The inlet fittings may be hot enough to cause burns. Remove the septum retainer nut, using the wrench if the nut is hot or sticks.
5.4.2.2.3. Remove the old septum or Merlin microseal, using tweezers if necessary. Be sure that it is completely removed and take care to avoid gouging or scratching the interior of the septum head.
5.4.2.2.4. Press a new septum or Merlin microseal into place firmly.
5.4.2.2.5. Replace the septum or Merlin microseal retainer nut, tightening it finger-tight until the c-ring is about 1 mm above the nut. Avoid overtightening.
5.4.2.2.6. Using MassHunter load a method to return the GC to appropriate settings. If prompted, do not save any method changes.
5.4.2.2.7. Allow the GC to return to the setpoints.

5.4.2.3. Record on the GCMS Maintenance Log.
5.4.2.3.1. Indicate on the log the type of maintenance performed. (Septum, Merlin, etc)

5.4.2.4. Required post-maintenance check: Successful daily QCC.

5.4.3. Syringe

5.4.3.1. Inspect monthly for cleanliness and ease of movement. Replace yearly, at a minimum, when in use.
5.4.3.1.1. Select [Maintenance], [Perform Maintenance], [Replace Syringe], [Start Maintenance].
5.4.3.1.2. Mount the injector on a parking post.
5.4.3.1.3. Open the injector door.
5.4.3.1.4. Slide the syringe carriage to the top position.
5.4.3.1.5. Completely loosen the plunger thumb screw until it reaches the stop, and then lift the plunger carrier off of the syringe plunger.
5.4.3.1.6. Open the syringe latch by swinging it in a counterclockwise direction.
5.4.3.1.7. Carefully pull the top of the syringe out of the flange guide, then lift the needle out of the needle support foot.
5.4.3.1.8. Carefully pass the new syringe needle through the guide hole in the needle support foot.
5.4.3.1.9. Align the syringe flange with the flange guide and press the syringe into place, keeping the needle end in the guide hole of the needle support foot. Make sure that the flat edge of the syringe flange faces out.
5.4.3.1.10. Close the syringe latch by swinging it clockwise until it snaps in place.
5.4.3.1.11. Slide the plunger carrier down until it is completely over the syringe plunger, and then tighten the plunger thumb screw until finger-tight.

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5.4.3.1.12. Manually move the plunger carrier up and down. If the syringe plunger does not move along with the carrier, repeat the previous steps until installed correctly. Be sure the plunger thumb screw is secure and tight. Verify that the needle is inside the guide hole of the needle support foot. The needle should be straight and pass freely through the needle guide hole. If the needle is bent or is outside the guide hole, remove the syringe and reinstall.

5.4.3.1.13. Close the injector door.

5.4.3.1.14. Mount the injector on the inlet.

5.4.3.2. Record on the GCMS Maintenance Log.

5.4.3.3. Required post-maintenance check: None.

5.4.4. Liner

5.4.4.1. Replace monthly, at a minimum, when in use.

5.4.4.1.1. Select [Maintenance], [Perform Maintenance], [Replace Liner], [Start Maintenance].

5.4.4.1.2. Be careful - The inlet fittings may be hot enough to cause burns. Flip the inlet open.

5.4.4.1.3. Remove liner with tweezers, being careful not to break the liner.

5.4.4.1.4. Hold the new liner with tweezers or lint free tissue and ensure the o-ring is on the liner about 2 to 3 mm from its top end.

5.4.4.1.5. Insert the liner straight down into the inlet and press gently to ensure it is seated.

5.4.4.1.6. Replace the inlet cover and slide the locking tab to the back.

5.4.4.1.7. Using MassHunter load a method to return the GC to appropriate settings. If prompted, do not save any method changes.

5.4.4.1.8. Allow the GC to return to the setpoints.

5.4.4.2. Record on the GCMS Maintenance Log.

5.4.4.3. Required post-maintenance check: Successful daily QCC.

5.4.5. Clean Source

5.4.5.1. Clean annually, at a minimum.

5.4.5.1.1. Vent the MSD by selecting [Instrument], [MS Vacuum Control], [Vent] in MassHunter software. Allow the vent cycle to run. When the cycle is complete and the temperatures are below 100 degrees Celsius, turn off the MSD.

5.4.5.1.2. Select [Maintenance], [Perform Maintenance], [Maintenance Mode], [Start Maintenance]. When the temperature reaches the setpoint turn the GC off.

5.4.5.1.3. Open the vent valve.

5.4.5.1.4. Detach the ribbon cables from the circuit board on the MSD chamber door.

5.4.5.1.5. Pull open the MSD chamber door by hand.

5.4.5.1.6. Detach the leads from the ion source, loosen the screws and remove the ion source.

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5.4.5.1.7. Remove the filaments using a hex ball driver.
5.4.5.1.8. Separate the repeller assembly from the source body. The repeller assembly includes the source heater assembly, repeller, and related parts.
5.4.5.1.9. Remove the repeller.
5.4.5.1.10. Unscrew the interface socket. A 10-mm open-end wrench fits the flats on the interface socket.
5.4.5.1.11. Remove the set screws for the lenses.
5.4.5.1.12. Push the lenses out of the source body.
5.4.5.1.13. If insulators are dirty, clean them with a cotton swab dampened with reagent-grade methanol. If that does not clean the insulators, replace them. Do not abrasively or ultrasonically clean the insulators.
If the extractor lens insulator is dirty, replace this item.
5.4.5.1.14. The filaments and source heater assembly cannot be cleaned ultrasonically. Replace these components if major contamination occurs.
5.4.5.1.15. Collect the following parts that contact the sample or ion beam to be cleaned.
5.4.5.1.15.1. Repeller
5.4.5.1.15.2. Source body
5.4.5.1.15.3. Repeller Block
5.4.5.1.15.4. Extractor Lens
5.4.5.1.15.5. Ion focus lens
5.4.5.1.15.6. Entrance lens
5.4.5.1.16. Abrasively clean the surfaces that contact the sample or ion beam.
5.4.5.1.17. Use an abrasive slurry of alumina powder and methanol on a cotton swab. Use enough force to remove all discolorations. Polishing the parts is not necessary; small scratches will not harm performance. Also abrasively clean the discolorations where electrons from the filaments enter the source body.
5.4.5.1.18. Rinse away all abrasive residue with reagent-grade methanol.
5.4.5.1.19. Make sure all abrasive residue is rinsed way before ultrasonic cleaning. If the methanol becomes cloudy or contains visible particles, rinse again.
5.4.5.1.20. Separate the parts that were abrasively cleaned from the parts that were not abrasively cleaned.
5.4.5.1.21. Ultrasonically clean the parts (each group separately) in polar and non-polar solvents; as recommended by the instrument manufacturer.
5.4.5.1.22. Place the parts in a clean beaker.
5.4.5.1.23. If needed, dry the cleaned parts in an oven at 100 °C for 5–6 minutes.
5.4.5.1.24. If needed, let the parts cool before you handle them.

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5.4.5.1.25. Take care to avoid re-contaminating cleaned and dried parts. Put on new, clean gloves before handling the parts. Do not set the cleaned parts on a dirty surface. Set them only on clean, lint-free cloths.
5.4.5.1.26. Slide the extractor lens into the source body.
5.4.5.1.27. Assemble the ion focus lens, entrance lens, and lens insulators.
5.4.5.1.28. Slide the assembled parts into the source body.
5.4.5.1.29. Install the set screws that hold the lenses in place.
5.4.5.1.30. Reinstall the repeller, repeller insulators, washer, and repeller nut into the source heater assembly. The resulting assembly is called the repeller assembly.
5.4.5.1.31. Reconnect the repeller assembly to the source body. The repeller assembly includes the source heater assembly, repeller, and related parts.
5.4.5.1.32. Reinstall the filaments, replace if excessively worn.
5.4.5.1.33. Do not overtighten the repeller nut or the ceramic repeller insulators will break when the source heats up. The nut should only be finger-tight.
5.4.5.1.34. Reinstall the ion source into the MSD and reattach the leads.
5.4.5.1.35. Close the vent valve.
5.4.5.1.36. Push the MSD chamber door closed and reattach the ribbon cables to the circuit board.
5.4.5.1.37. Power on the GC.
5.4.5.1.38. Holding the MSD chamber door closed, power on the MSD and ensure that the turbo pump speed climbs to 100%.
5.4.5.1.39. Use the MassHunter software to reconnect and apply setpoints.
5.4.5.1.40. Allow the instrument to equilibrate for two hours prior to tuning.
5.4.5.2. Record on the GCMS Maintenance Log.
5.4.5.3. Required post-maintenance check: Successful daily QCC and monthly QCC on all approved methods.

5.4.6. Gold Seal

5.4.6.1. Replace annually, at a minimum.
5.4.6.1.1. Select [Maintenance], [Perform Maintenance], [Replace Gold Seal], [Start Maintenance]. Vent the MSD by selecting [Instrument], [MS Vacuum Control], [Vent] in MassHunter. Allow the vent cycle to run. When the cycle is complete and the temperatures are below 100 degrees Celsius, turn off the MSD.
5.4.6.1.2. Be careful - The inlet fittings may be hot enough to cause burns. Loosen the inlet column nut with the ¼ inch wrench and remove the column from the inlet. Cap the open end of the column to prevent contamination.
5.4.6.1.3. Remove the insulation cup from around the base of the inlet using the star screwdriver.
5.4.6.1.4. Use the 1/2-inch wrench to loosen the reducing nut, and then remove it.

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5.4.6.1.5. The washer and seal are inside the reducing nut. Remove them, noting their orientation.
5.4.6.1.6. Handle the new gold seal and washer with clean, lint-free, non-nylon gloves. Place the washer in the reducing nut. Place the new inlet base seal on top of it with the raised portion facing down.
5.4.6.1.7. Replace the reducing nut. Use the 1/2-inch wrench to tighten the nut.
5.4.6.1.8. Replace the column and the insulation cup.
5.4.6.1.9. Allow the GC to return to the setpoints.
5.4.6.1.10. Ensure that the vent valve is closed. Holding the MSD chamber door closed, power on the MSD and ensure that the turbo pump speed climbs to 100%.
5.4.6.1.11. Use the MassHunter software to reconnect and apply setpoints.
5.4.6.1.12. Allow the instrument to equilibrate for two hours prior to tuning.
5.4.6.2. Record on the GCMS Maintenance Log.
5.4.6.3. Required post-maintenance check: Successful daily QCC and monthly QCC on all approved methods.

5.4.7. Gas Filter

5.4.7.1. Replace as needed. When in-use, filter changes from green to brown indicating air and/or water leak.
5.4.7.1.1. Select [Maintenance], [Perform Maintenance], [Gas Clean Filter Maintenance], [Start Maintenance].
5.4.7.1.2. Unscrew the ring nut that secures the filter and remove the used carrier gas filter from the base.
5.4.7.1.3. Remove the old upper set of O-rings and replace with new O-rings.
5.4.7.1.4. Remove the two plugs from the bottom of the new filter and install the new gas filter onto the base.
5.4.7.1.5. Re-install and tighten the ring nut. Allow the ring nut to pull the filter tight onto the connecting unit.
5.4.7.2. Record on the GCMS Maintenance Log
5.4.7.3. Required post-maintenance check: Successful daily QCC.

5.4.8. Split Vent Trap

5.4.8.1. Replace yearly, at a minimum.
5.4.8.1.1. Select [Maintenance], [Perform Maintenance], [Replace Split vent trap], [Start Maintenance].
5.4.8.1.2. Remove the top cover. If applicable, loosen the screw that hold the split vent valve in place and rotate the retaining clip out of the way.
5.4.8.1.3. The trap is located inside of the welding. Loosen the large knob counterclockwise to access the trap.
5.4.8.1.4. Lift the trap assembly and replace the new trap.

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5.4.8.1.5. Reassemble split vent trap and attach the top cover.
5.4.8.2. Record on the GCMS Maintenance Log.
5.4.8.3. Required post-maintenance check: Successful daily QCC.

6. Procedure

6.1. Instrument Settings

6.1.1. Select a GC-MS method based on the sample and any analysis results.

6.1.1.1. The SCREEN2 (SCRN2) method shall be used when a controlled substance is not previously indicated and a GC-MS analysis is performed, i.e. negative preliminary testing and/or infrared analysis indicated a non-controlled substance, and a GC-MS analysis is performed.

6.1.1.2. The SCREEN2 (SCRN2) method shall be used for at least one sample preparation when GC-MS is the sole technique used in analysis.

6.1.1.3. Each method may be used with split ratios of 5:1, 20:1, or 100:1. Numbers in front of the method name indicates the split ratio.

6.1.1.4. Each method shall wash the syringe at least 10 times between injections to ensure sample integrity.

6.1.1.5. When the standard methods are not appropriate to analyze a compound, a modified method may be used in accordance with the Wake County Bureau of Forensic Services Standard Operating Procedure for Exceptions.

6.1.2. LOW3 – typically used for compounds that elute prior to 15 minutes in the screen method, e.g. cocaine, amphetamines, some steroids, some synthetic cannabinoids, most opiates and most benzodiazepines.

6.1.2.1. 1.5 minutes initial time
6.1.2.2. 100 °C initial temperature
6.1.2.3. 30 °C/minute ramp
6.1.2.4. 300 °C final temperature
6.1.2.5. 6.83 minutes final time
6.1.2.6. 15.0 minutes total time
6.1.2.7. Scan range = 40-600 amu
6.1.2.8. 250 °C source temperature
6.1.2.9. 150 °C quadrupole temperature

6.1.3. SCREEN2 (SCRN2) – Use this method when GC-MS is used to screen samples to determine if a controlled substance may be present.

6.1.3.1. 1.5 minutes initial time
6.1.3.2. 100 °C initial temperature
6.1.3.3. 30 °C/minute
6.1.3.4. 300 °C final temperature
6.1.3.5. 26.83 minutes final time

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6.1.3.6. 35.0 minutes total time
6.1.3.7. Scan range = 40-600 amu
6.1.3.8. 250 °C source temperature
6.1.3.9. 150 °C quadrupole temperature

6.1.4. ISOTHERMAL3 (ISO3) – This method can be used to differentiate structurally similar compounds including phenethylamines and fentanyl derivatives such as ortho, meta, and para-Fluoroisobutyryl Fentanyl, ortho, meta, and para-Fluorobutyryl Fentanyl, Cyclopropyl Fentanyl, and Crotonyl Fentanyl.

6.1.4.1. 25.00 minutes initial time
6.1.4.2. 250 °C initial temperature
6.1.4.3. 250 °C final temperature
6.1.4.4. 25.0 minutes total time
6.1.4.5. Scan range = 40-600 amu
6.1.4.6. 250 °C source temperature
6.1.4.7. 150 °C quadrupole temperature

6.1.5. CANNABINOID2 (CANN2) – Use this method to differentiate structurally similar cannabinoids to include delta-9-tetrahydrocannabinol (THC) and delta-9-tetrahydrocannabinolic acid.

6.1.5.1. 0.00 minutes initial time
6.1.5.2. 260 °C initial temperature
6.1.5.3. 7°C/Minute ramp
6.1.5.4. 300 °C final temperature
6.1.5.5. 4.29 minutes final time
6.1.5.6. 10.001 minutes total time
6.1.5.7. Scan range = 40-600 amu
6.1.5.8. 250 °C source temperature
6.1.5.9. 150 °C quadrupole temperature

6.1.6. SLOW2 – This method can be used to differentiate structurally similar compounds including N-Ethyl Pentylone, 3,4-Methylenedioxy-alpha-isopropylaminobutiophenone, 3,4-Methylenedioxy-alpha-propylaminobutiophenone, and N-methyl-N-propyl Methylone.

6.1.6.1. 1.5 minutes initial time
6.1.6.2. 100 °C initial temperature
6.1.6.3. 30°C/Minute ramp
6.1.6.4. 180 °C intermediate temperature
6.1.6.5. 7°C/Minute ramp
6.1.6.6. 300 °C final temperature
6.1.6.7. 3.69 minutes final time
6.1.6.8. 25.00 minutes total time

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6.1.6.9. Scan range = 40-600 amu
6.1.6.10. 250 °C source temperature
6.1.6.11. 150 °C quadrupole temperature

6.2. Shutdown / Startup

6.2.1. The GC-MS shall be left on at all times.
6.2.2. The computer may be shut down or restarted if needed.
6.2.3. A successful daily QCC check must be performed following any GC or MS shutdown.
6.2.4. Record any shutdown on the GC-MS activity Log.

6.3. Prior to the injection of a sample, perform a negative QCC injection using the negative quality control extraction prepared using the same techniques, materials, reagents and solvents as the sample preparation. Use the same method and split ratio as the sample.

6.3.1. Prepare the negative QCC extraction at the time of sample preparation from the same solvent source used in sample preparation.
6.3.2. Evaluate the negative QCC to ensure that the instrument and solvent are free of any controlled substance, any substance being identified in the sample and any substance that may interfere with the identification of sample component(s).
6.3.2.1. Note the presence of large amounts of common gas chromatography peaks (e.g., siloxanes) in the GC-MS activity log and notify the Drug Chemistry Technical Leader.
6.3.2.2. Record all negative QCC's results (pass/fail) and any comments on the GC-MS activity log.

6.4. Evaluate and prepare samples prior to injection to avoid overloading, extreme pH, oils, sugars and compounds known to be retained in the instrument.

6.4.1. At a minimum, filter solid samples with solvent to prevent particulate matter and undesired compounds from being introduced into the instrument (e.g., sugars). Particulate matter should not be visible in an autosampler vial.
6.4.2. Refer to the Drug Chemistry Unit Technical Procedure for Extractions for additional sample preparation.
6.4.3. Extract/convert sulfates prior to introduction into the instrument.

6.5. Use the current date in the names of sequences. Sequences need not be archived.

6.6. Include the Wake County Bureau of Forensic Services case file number in the data file name and any additional information needed to uniquely identify the sample.

6.6.1. Data files associated with casework and performance checks shall not be deleted or overwritten.
6.6.2. Notify the Drug Chemistry Technical Leader if the data storage location becomes full.

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6.7. The GC-MS provides retention time data and mass spectral data. Refer to the Drug Chemistry Unit Technical Procedure for Drug Chemistry Analysis. Evaluate the chromatogram and spectra for each peak.

6.8. Record in the case file the:

6.8.1. Total Ion Chromatogram (TIC) for the corresponding blank.

6.8.2. Sample TIC.

6.8.3. Mass spectra of significant peaks or a notation in the case file indicating that these peaks have been checked. Peaks may be deemed significant based on their abundance and/or their identity or controlled status.

6.8.4. Expanded mass spectra of any confirmed phenethylamines, nitazines, and any compound where the expansion is necessary in order to view the spectral pattern of the substance.

6.9. Mass Spectral Comparison

6.9.1. For a positive mass spectral comparison, the sample mass spectrum must be substantially comparable, i.e., equivalent, to that of primary or secondary reference material.

6.9.1.1. Record in the case file the mass spectrum of the reference material with the supplier and lot number or other Drug Chemistry Unit designation. Library search results may be included.

6.9.2. If a derivatizing agent is used, the mass spectrum of the sample must be compared to published spectral data from a published reference generally accepted in the field and found to be substantially comparable, i.e., equivalent.

6.9.2.1. Record in the case record the mass spectrum of the reference material with the supplier and lot number or other Drug Chemistry Unit designation and the supplier and lot number of the derivatizing agent.

6.10. GC Retention Time (RT) Comparison

6.10.1. For a positive GC RT comparison of compounds with a retention time of 10 minutes or less, the difference between the sample retention time and a primary or secondary reference material retention time must be 0.10 minute or less. For a positive GC RT comparison of compounds with a retention time greater than 10 minutes, the percent difference between the sample retention time and a primary or secondary reference material retention time must be 1.0% or less.

6.10.1.1. The chromatographic peaks must be visually smooth and symmetrical.

6.10.1.2. The reference material must be run within thirty days before or after the case sample.

6.10.1.2.1. If the reference material is a component of the monthly QCC solution, the retention time may be used for the month in which it was run plus the first seven calendar days of the following month.

6.10.1.2.2. There must not be any column maintenance performed between the analysis of the sample and reference material.

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6.10.1.3. Record in the case file:
6.10.1.3.1. Reference material TIC with the retention time(s) displayed.
6.10.1.3.2. Reference material mass spectrum and any other significant peaks with the retention time(s) displayed.
6.10.1.3.3. Reference material standard supplier and lot number or other Drug Chemistry Unit designation.
6.10.1.3.4. The percent difference of the reference material and sample retention times, rounded to one decimal place.

7. Calculations

7.1. Percent Difference Calculation, round to one decimal place:
7.1.1. |(reference material RT – sample RT)| / (reference material RT) * 100

8. Limitations

8.1. The GC-MS methods described in this procedure cannot be used to distinguish between optical isomers.
8.1.1. Discretion shall be used when using GC-MS analysis for other isomer determination.
8.2. Introduction of improperly prepared samples may lead to poor sensitivity and carryover.
8.3. The ISOTHERMAL2 temperature program alone cannot be used to distinguish retention times in a mixture containing Methoxyacetyl Fentanyl and Cyclopropyl Fentanyl.
8.4. The SLOW method alone cannot be used to distinguish retention times in a mixture containing N,N-Dimethylpentylone and N-methyl-N-propyl Methylone.

9. Safety

9.1. Handle syringes with care to avoid punctures.
9.2. Use extreme caution handling/installing/removing/transporting compressed gas cylinders. Cylinders shall not be moved without the cylinder cap securely in place.
9.3. Gas Chromatograph and Mass Spectrometer may be extremely hot. Avoid touching hot areas and wear protective gloves while performing maintenance.

10. References

10.1. Agilent 8890 GC Instrument Manuals.
10.2. Agilent 5977 MSD Instrument Manuals.
10.3. Moffat, A. C., et al., eds. *Clarke's Isolation and Identification of Drugs.* 2nd Edition. London: Pharmaceutical Press, 1986.

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10.4. Moffat, A.C., et al., eds. *Clarke's Analysis of Drugs and Poisons.* 4rd Edition. London: Pharmaceutical Press, 2011.
10.5. Skoog, Douglas A., F. James Holler and Timothy A. Nieman. *Principles of Instrumental Analysis*. 5th Edition. Garcourt Brace & Company, 1998.
10.6. *Agilent GC-MSD ChemStation and Instrument Operation Student Manual Course Number H4043A Volume 1*, Revision E.02.xx. Agilent Technologies: printed February 2008.
10.7. *Guide for the Use of the International System of Units (SI).* NIST Special Publication 811, 2008 Ed., (March 2008; 2nd printing November 2008). P.43.

11. Records

11.1. GC-MS Maintenance Log
11.2. GC-MS Daily QCC Log
11.3. GC-MS Monthly QCC Log
11.4. GC-MS Activity Log

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Document Revision History		
Revision Date	Prepared By	Revision
04/03/2023	LW	Removed result requirements for activity log in 4.1.1.1. Updated 6.1.6. Cannabinoid method Removed 6.6.2. data archive requirements Corrected typos throughout document
05/04/2023	LW	Revised 4.7. Annual/Monthly Quality Control Check Added 6.1.7. Slow Method Added 8.4 Limitation
02/21/2024	LW	3.1. Equipment: updated model number and added MassHunter Software 3.3. Materials: removed liner O-ring, pump oil, funnel 5. Maintenance: removed requirement to document column trim amount in the activity log 5.4. Suggested Routine Maintenance: revised frequency of filling and rinsing for wash vials, revised button command instructions to new touchscreen commands for all maintenance, removed pump oil maintenance, replaced drawout plate and drawout cylinder with extractor lens, updated gas filter steps. 6.1. Instrument Settings: removed splitless (nosplit) method, removed HIGH method, updated source temperature to 250C Updated throughout procedure for grammar and clarity. Updated revision history, header, and title formatting.
11/7/2024	A. Abernethy	Document revised to reflect the agency name change from Raleigh/Wake City-County Bureau of Identification to Wake County Bureau of Forensic Services, effective December 1, 2024. No change to procedure content.
4/3/2025	L. Wiley	6.1. Instrument Settings – updated method version and scan range to 600 amu for Low, Screen, Isothermal, Cannabinoid, and Slow methods.
03/23/2026	L. Wiley	4.7. Annual/Monthly Quality Control Check – updated 4.7.3. and 4.7.4. to current method versions.

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		6. Procedure – updated current method version in 6.1.1.1. and 6.1.1.2. and updated MS casefile requirements in 6.8.3. and 6.8.4.

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