CORNERSTONE LABORATORIES LLC

1775 Moriah Woods Blvd., Ste. 12 • Memphis, TN 38117 ▪ (901) 398-4001 Fax: (901) 398-4223

Ethanol by Headspace GC/MS

Standard Operating Procedure

Reviewed By: _____ Laboratory Director

Reviewed By: _____ Office Manager

Approved by: _____ President/Technical Director

Table of Contents

1.0 PURPOSE

The purpose of this standard operating procedure is to describe the protocols used to extract and analyze Ethanol contained in Kombucha drinks.

2.0 APPLICABLE MATRIX

2.1 Bottled Kombucha Drinks.

3.0 METHOD DETECTION (MDL)

3.1 The MDLs were established using procedures given in EPA 40 CFR Part 40, Appendix B.

3.2 Seven replicate samples are analyzed at a concentration 3 to 5 times higher than the lowest point on the analytical curve.

3.3 The MDL is the concentration of analyte at which there is analyte present with 99% confidence.

3.4 The results of the seven replicate data sets are tabulated and the statistical calculations are performed electronically on at least a semi-annual basis to establish or update the MDL's and MQLs for each analyte and matrix.

4.0 SCOPE AND APPLICATION, INCLUDING COMPONENTS TO BE ANALYZED.

4.1 This method details the analysis of purgeable organic compounds in water based matrices by Headspace/ Gas Chromatography/Mass Spectrometry. The procedures describe the operational details needed to perform analysis at Cornerstone Laboratories.

4.2 The following compound may be determined by this method:

Ethanol

5.0 SUMMARY OF TEST METHOD

5.1 The determination of Ethanol by this method is a two-step process where the sample is first introduced by Purge & Trap and then analyzed by gas chromatography/mass spectrometry.

5.2 Sample Introduction Method: The Ethanol is introduced into the Gas Chromatograph by the EPA Headspace Method 5030B.

5.3 Quantitation Method: All components are separated *via* gas chromatography and detected using a mass spectrometer, which provides both qualitative and quantitative information. The described quantitative method is based on EPA Method 624 Purgeables, Part 136, Title 40 and EPA Method 8260B, SW-846. The procedures described here are designed to meet or exceed the criteria from both of these EPA quantitative methods. This is accomplished by meeting the more stringent requirement from both methods. Please refer to the EPA methods for additional details.

6.0 DEFINITIONS

Analyst: the designated individual who performs the "hands-on" analytical methods and associated techniques and who is the one responsible for applying required laboratory practices and other pertinent quality controls to meet the required level of quality.

Batch: environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A **preparation batch** is composed of one to 20 environmental samples of the same NELAC-defined matrix, meeting the above-mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An **analytical batch** is composed of prepared environmental samples (extracts, digestates or concentrates) which are analyzed together as a group. An analytical batch can include prepared samples originating from various environmental matrices and can exceed 20 samples. (NELAC Quality Systems Committee)

Blank: a sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. Blanks include Equipment Blank: a sample of analyte-free media, which has been used to rinse common sampling equipment to check effectiveness of decontamination procedures. Field Blank: blank prepared in the field by filling a clean container with pure de-ionized water and appropriate preservative, if any, for the specific sampling activity being undertaken. (EPAOSWER) Instrument Blank: a clean sample (e.g., distilled water) processed through the instrumental steps of the measurement process; used to determine instrument contamination. Method Blank: a sample of a matrix similar to the batch of associated samples (when available) that is free from the analytes of interest and is processed simultaneously with and under the same conditions as samples through all steps of the analytical procedures, and in which no target analytes or interferences are present at concentrations that impact the analytical results for sample analyses. Reagent Blank: (method reagent blank): a sample consisting of reagent(s), without the target analyte or sample matrix, introduced into the analytical procedure at the appropriate point and carried through all subsequent steps to determine the contribution of the reagents and of the involved analytical steps.

Certified Reference Material (CRM): a reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO Guide 30 - 2.2)

Corrective Action: the action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence.

Demonstration of Capability: a procedure to establish the ability of the analyst to generate acceptable accuracy.
Holding Times (Maximum Allowable Holding Times): the maximum times that samples may be held prior to analysis and still be considered valid or not compromised. (40 CFR Part 136)
Inspection: an activity such as measuring, examining, testing, or gauging one or more characteristics of an entity and comparing the results with specified requirements in order to establish whether conformance is achieved for each characteristic.
Laboratory Control Sample (however named, such as laboratory fortified blank, spiked blank, or QC check sample): a sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes or a material containing known and verified amounts of analytes. It is generally used to establish intra-laboratory or analyst specific precision and bias or to assess the performance of all or a portion of the measurement system.
Laboratory Duplicate: aliquots of a sample taken from the same container under laboratory conditions and processed and analyzed independently.
Matrix: the substrate of a test sample.
Matrix Spike (spiked sample or fortified sample): a sample prepared by adding a known mass of target analyte to a specified amount of matrix sample for which an independent estimate of Target analyte concentration is available. Matrix spikes are used, for example, to determine the effect of the matrix on a method's recovery efficiency.
Matrix Spike Duplicate (spiked sample or fortified sample duplicate): a second replicate matrix spike prepared in the laboratory and analyzed to obtain a measure of the precision of the recovery for each analyte.
Precision: the degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms.
Preservation: refrigeration and/or reagents added at the time of sample collection (or later) to maintain the chemical and/or biological integrity of the sample.
Quality Control: the overall system of technical activities whose purpose is to measure and control the quality of a product or service so that it meets the needs of users.
Quality Control Sample: an uncontaminated sample matrix spiked with known amounts of analytes from a source independent from the calibration standards. It is generally used to establish intra-laboratory or analyst specific precision and bias or to assess the performance of all or a portion of the measurement system.
Quantitation Limits: levels, concentrations, or quantities of a target variable (e.g., target analyte) that can be reported at a specified degree of confidence.
Range: the difference between the minimum and the maximum of a set of values.

Reference Material: a material or substance one or more properties of which are sufficiently well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.
Reference Method: a method of known and documented accuracy and precision issued by an organization recognized as competent to do so.
Reference Standard: a standard, generally of the highest meteorological quality available at a given location, from which measurements made at that location are derived. (VIM-6.08)
Replicate Analyses: the measurements of the variable of interest performed identically on two or more sub-samples of the same sample within a short time interval.
Spike: a known mass of target analyte added to a blank sample or sub-sample; used to determine recovery efficiency or for other quality control purposes. (NELAC)
Standard: the document describing the elements of laboratory accreditation that has been developed and established within the consensus principles of NELAC and meets the approval requirements of NELAC procedures and policies. (ASQC)
Standard Operating Procedures (SOPs): a written document which details the method of an operation, analysis or action whose techniques and procedures are thoroughly prescribed and which is accepted as the method for performing certain routine or repetitive tasks. (QAMS)
Standardized Reference Material (SRM): a certified reference material produced by the U.S. National Institute of Standards and Technology or other equivalent organization and characterized for absolute content, independent of analytical method. (EPA-QAD)
Supervisor (however named): the individual(s) designated as being responsible for a particular area or category of scientific analysis. This responsibility includes direct day-to-day supervision of technical employees, supply and instrument adequacy and upkeep, quality assurance/quality control duties and ascertaining that technical employees have the required balance of education, training and experience to perform the required analyses. (NELAC)
Surrogate: a substance with properties that mimic the analyte of interest. It is unlikely to be found in environment samples and is added to them for quality control purposes. (QAMS)
Test Method: an adoption of a scientific technique for a specific measurement problem.

7.0 INTERFERENCE

Major contaminant sources are volatile materials in the laboratory and impurities in the inert purging gas and in the sorbent trap. The use of non-polytetrafluoroethylene (PTFE) thread sealant, plastic tubing, or flow controllers with rubber components should be avoided, since such materials outgas organic compounds which will be concentrated in the trap during the purge operation. Analyses of calibration and reagent blanks provide information about the presence of contaminants. When potential interfering peaks are

noted in blanks, the analyst should change the purge gas source and regenerate the molecular sieve purge gas filter. Subtracting blank values from sample results is not permitted

7.1 Contamination may occur when a sample containing low concentrations of volatile organic compounds is analyzed immediately after a sample containing high concentrations of volatile organic compounds. After the analysis of a sample containing high concentrations of volatile organic compounds, one or more blanks should be analyzed to check for cross-contamination. Alternatively, if the sample immediately following the high concentration sample does not contain the volatile organic compounds present in the high level sample, freedom from contamination has been established.

7.2 For samples containing large amounts of water-soluble materials, suspended solids, high boiling compounds, or high concentrations of compounds being determined, it may be necessary to wash the purging device with a soap solution, rinse it with organic-free reagent water, and then dry the purging device in an oven at 105°C. In extreme situations, the entire purge-and-trap device may require dismantling and cleaning. Screening of the samples prior to purge-and-trap GC/MS analysis.

8.0 SAFETY

8.1. Review the Laboratory Safety SOP Manual and the Contingency Plans and Emergency Procedures for a Hazardous Waste Generator**.**

8.2. Safety equipment must be used when appropriate and must be maintained in good operating condition. Safety glasses, long pants, and closed-end shoes must be worn in the Laboratory at all times.

8.3. Use **CAUTION** with strong irritants such as acids and bases. Avoid breathing the fumes of these irritants by using them in a hood when possible and keeping the face away from open containers of these chemicals. Avoid contact of these irritants with skin and clothing by appropriate use of gloves, apron, face mask and hood shield.

9.0 EQUIPMENT AND SUPPLIES

9.1 The operation, cleaning and scheduled maintenance procedures prescribed by the equipment manufacture are followed as provided in the Operator's Manuals. Maintenance contracts, provided by the manufacture, are purchased for the Gas Chromatograph/Mass Spectrometer Systems and Sample Screening System. Documentation of cleaning, maintenance or system modifications are recorded in a maintenance logbook which accompanies each instrument system.

9.2Syringes:

9.2.1 Hamilton Micro liter/Gastight Syringes: 5 µL, 10 µL, 50 µL, 100 µL, 500 µL, 5 mL. The Hamilton syringes are delicate and must be handled with care. The syringes are cleaned by 2 to 3 rinses with methanol. The Hamilton syringes must never be heated in an oven or damage may result. Damaged or corroded syringes must be discarded.

9.2.2 B-D Brand Multifit Reusable Syringe: 5 mL. The B-D Brand Multifit syringe is used only in sample screening or transfer applications. This syringe must not be used for the measurement of precise volumes. The B-D syringe is more rugged and can be baked in a low temperature oven at less than 120 °C.

9.3 Volumetric Flasks (class A) and Graduated Cylinders: 10 mL, 25 mL, 50 mL, 100 mL.

9.3.1 The volumetrics are equipped with a penny head ground glass stopper.

9.3.2 The volumetric flasks and graduated cylinders are cleaned by rinsing with methanol and laboratory reagent water. The volumetrics are dried in a low temperature oven at less than 120 °C. Never use a brush or strong alkali solution to clean the volumetrics.

9.4 Glass Sample (VOA) and Standard Vials:

9.4.1 40 mL VOA vials with a Teflon□/silicone septa and polypropylene open-top cap.

9.4.2 2 mL vials with Teflon□/silicone/Teflon□ septa and polypropylene open-top cap.

9.5 Fisher Optima Grade Water.

9.6 Dispatch Forced-Air Oven #POV0005.

9.7 Delta Range/Mettler Top-loading Balance, capable of weighing 0.001 g #RVL0003.

9.8 Sartorius T-Base Analytical Balance, capable of weighing 0.0001 g #RBL0002.

9.9 pHydrion pH Paper.

9.10 Refrigerator/Freezer, Lab-Line, Model.

9.11 Gas Chromatograph/Mass Spectrometer VOCMSD System 1:

9.11.1 Perkin-Elmer Auto system XL Gas Chromatograph.

9.11.2 Perkin-Elmer Turbo mass Gold Mass Spectrometer.

9.11.3 OI Analytical 4560 Headspace Sample Concentrator.

9.11.4 OI Analytical 4552 Auto sampler.

9.11.5 Dell Pentium-III Personal Computer.

9.11.6 Perkin-Elmer Turbo mass Computer Software.

9.11.7 Restek VMS, 30 m x 0.25 mm ID capillary column, 1.4 µm film.

10.0 REAGENTS AND STANDARDS

10.1 Working with volatile compounds presents a number of challenges not normally confronted in the handling of most other chemicals. Volatile compounds are easily lost from prepared solutions or, when present in the laboratory environment, can cross-contaminate other samples. Sources of the reagents and chemicals are given, but may change based on availability, quality and cost. The use of a different source is acceptable without modification of the procedures provided the products are equivalent.

10.2 Organic Solvents (Purge & Trap grade):

10.2.1 Methanol: CH3OH - Purge & Trap grade methanol is purchased in 500 mL amber bottles from Fisher Scientific.

10.3 Acids and Bases:

10.3.1 Hydrochloric Acid, HCL - Fisher Certified ACS Plus Grade

10.3.1.1 A 1:1 HCL solution used for sample preservation is prepared by making a 1:1 dilution of the HCl acid into laboratory reagent water.

10.4 Laboratory Reagent Water:

10.4.1 Fischer Scientific Optima Grade Water

10.5 Internal Standard and Surrogate Solutions:

10.5.1 250 ug/mL Internal Standard and Surrogate Standard Solutions: An internal standard and surrogate standard solutions are injected into every sample by the autosampler just prior to analysis. The internal standard compounds are **Chlorobenzene-d 5, 1,4-Dichlorobenzene-d4, 1,4-Difluorobenzene and Pentafluorobenzene..** The surrogate compounds are **Dibromofluoromethane, 4-Bromofluorobenzene, 1,2-Dichloroethane-d4 and Toluene-d8**. These compounds may be purchased in the prepared mixtures C-307 and C-440 from NSI Environmental Solutions at a concentration of 2500 µg/mL in methanol.

10.5.1.1 Dispense accurately 1.5 mL of C-307 and C-440 (or equivalent solution) into separate vials containing 13.5 mLs of methanol. The working internal standard and surrogate solution is stored at ≤ -20.0 °C in 25 mLvials. New working standard is prepared from fresh stock solutions every 6 months or before the expiration date of the stock standard, whichever comes first.

10.6 Calibration Standards:

10.6.1 The stock calibration standard used for the preparation of the initial calibration is purchased from NSI Environmental Solutions in methanol: C-350 (200µg/mL and W-0017 (1000 ug/mL of Ethanol diluted 1:5or 200ug/mL). These standard mixtures include all of the compounds routinely reported by this method. The stock solutions are ready to use as working standards, without dilution, after the stock solution ampoules are opened and the contents transferred to a labeled 2 mL vial with a Teflon-lined screw cap. Once opened, the working solutions must be used within 14 days or before the stock standard expires, whichever comes first. All stock and working standards are stored at ≤ -20.0 °C. A list of the normal calibration levels is given below where a minimum of 5 levels is selected for instrument calibration. Additional calibration levels may be prepared as deemed necessary. Additional analytes from the list of compounds found in Method 8260B may be added to this method once proficiency has been demonstrated through the validation exercise. The calibration standards must be prepared from the working standards just prior to use in a batch analysis. The calibration standard dilutions must be prepared with calibrated syringes and volumetrics. Mixing of the dilutions is

accomplished by gently rotating the volumetric flasks slowly 2 times (do not shake!). Transfer the calibration standards to zero headspace VOA vials immediately after preparation to minimize the loss of volatile compounds.

10.6.2 Initial Calibration Standards:

10.6.2.1 5ug/L C-350 calibration standard: add 1.0uL of the calibration standard into a 40 mL VOA Vial containing 35mL of laboratory reagent water. 5ug/L W-0017 calibration standard: add 1.0uL of the calibration standard into the same VOA vial containing 35 mL of laboratory reagent water. Dilute to final volume and cap vial. 10.6.2.2 10ug/L C-350calibration standard: add 2.0uL of each calibration standard into a 40 mL VOA Vial containing 35mL of laboratory reagent water. 10ug/L W-0017 calibration standard: add 2.0uL of the calibration standard into a 40 mL VOA Vial containing 35mL of laboratory reagent water. Dilute to final volume and cap vial. 10.6.2.3 25ug/L- C-350 calibration standard: add 5.0uL of the calibration standard into a 40 mL VOA Vial containing 35mL of laboratory reagent water. 25ug/L W-0017 calibration standard: add 5.0uL of the calibration standard into the same VOA vial containing 35 mL of laboratory reagent water. Dilute to final volume and cap vial.

10.6.2.4 50ug/L C-350 calibration standard: add 10.0uL of the calibration standard into a 40 mL VOA Vial containing 35mL of laboratory reagent water. 50ug/L W-0017 calibration standard: add 10.0uL of the calibration standard into the same VOA vial containing 35 mL of laboratory reagent water. Dilute to final volume and cap vial

10.6.2.5 75ug/L C-350 calibration standard: add 15.0uL of the calibration standard into a 40 mL VOA Vial containing 35mL of laboratory reagent water. 75ug/L W-0017 calibration standard: add 15.0uL of the calibration standard into the same VOA vial containing 35 mL of laboratory reagent water. Dilute to final volume and cap vial 10.6.2.6 100ug/L C-350 calibration standard: add 20.0uL of the calibration standard into a 40 mL VOA Vial containing 35mL of laboratory reagent water. 100ug/L W-0017 calibration standard: add 20.0uL of the calibration standard into the same VOA vial containing 35 mL of laboratory reagent water. Dilute to final volume and cap vial.

10.6.2.7 200ug/L C-350 calibration standard: add 40.0uL of the calibration standard into a 40 mL VOA Vial containing 35mL of laboratory reagent water. 200ug/L W-0017 calibration standard: add 40.0uL of the calibration standard into the same VOA vial containing 35 mL of laboratory reagent water. Dilute to final volume and cap vial

10.6.3 Calibration Check Standards:

10.6.3.1 These are the working calibration check compounds (CCCs) and system performance check compounds (SPCs) required by EPA method 8260B. This is a 2000ug/mL of Restek#30427 and Restek#30075 diluted 1:10 with methanol. These standards are a second source standard.

10.6.3.2 CCC - 50ug/L Restek#30427 standard: add 10.0uL of diluted standard into a 40 mL VOA Vial containing 35mL of laboratory reagent water. Dilute to final volume and cap flask.

10.6.3.3 SPC - 50ug/L Restek+ #30075 standard: add 10.0uL of the diluted standard into a 40 mL VOA Vial containing 35mL of laboratory reagent water. Dilute to final volume and cap flask.

10.6.3.4 Calibration checks for other compounds are prepared only for those analytical tests or samples, which require the special compounds.

10.7 Laboratory Control Standards and Matrix Spike Standards:

10.7.1 The compounds contained in the matrix spike standards are used for the preparation of Laboratory Control samples (LCS's) and sample matrix spikes. The following stock standard is purchased from NSI Environmental Solutions: C-193 (2500 µg/mL). The stock and working standards are stored at ≤ -20.0 °C. and the mixtures are used as second source standards.

10.7.2 The LCS and matrix spike standards must be prepared from the working standards just prior to use in a batch analysis. Standard dilutions must be mixed by gently rotating the volumetric flasks slowly 2 times (do not shake!). Transfer the standard solutions to zero headspace VOA vials immediately after preparation to minimize the loss of volatile compounds.

10.7.3 Matrix Spike/Laboratory Control Standard: NSI Environmental Solutions: C-193 (2500 µg/mL) matrix spike standard. This solution must be used within 6 months or before the expiration of the standard, whichever comes first.

10.7.4 Laboratory Control Sample Preparation: 50ug/L NSI Environmental Solutions: C-193 (2500 µg/mL) matrix spike/lab control standard: add 2.0uL of matrix spike/lab control standard into a 100 mL volumetric flask containing 90 mL of laboratory reagent water. Dilute to final volume and cap flask. This solution must be used within 6 months or before the expiration of the standard, whichever comes first.

10.7.5 Matrix Spike Preparation: A 50 ug/L matrix spike is prepared from the 2500ug/L matrix spike/lab control standard for every 10 samples. Duplicate matrix spikes prepared for every 20 samples meets the 10% requirement. The matrix spike is prepared by transferring the prepared standard into one of the 40 mL VOA sample vials. Multiple measurements of the VOA vials used by the laboratory has demonstrated that the volume of the VOA vials is approximately 40 ± 0.5 mL. Volatile loss is minimized

by directly transferring refrigerated standards to refrigerated sample vials. Comparing the recovery of the LCS and matrix spike duplicates can monitor variation in the vial volume.

11.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

11.1 All samples must be kept under refrigeration at all times prior to analysis. All samples must be analyzed prior to expiration dates.

11.2 Sample Storage:

11.5.1 Samples are stored in a segregated refrigerator for VOC's at 4.0 ± 2 °C until time for analysis.

12.0 QUALITY CONTROL

12.1 Quality control procedures for the operation of the GC/MS instruments include:

12.1.1 The GC/MS system must be tuned to meet the BFB specifications in **Table 1.**

12.1.2 Evaluation of retention time windows to ensure that all compounds are detected within their respective time windows and meet the ± 3.0 second requirement in **Section 14.3.1.3.2**

12.1.3 There must be an initial calibration of the GC/MS system as described in **Section 13.1**.

12.1.4 The GC/MS system must meet the CCC and SPCC criteria as described in **Section 13.1.6**.

12.1.5 A laboratory reagent water blank (method blank) must be analyzed before the samples in the analytical batch. The blank results must demonstrate that the analytical system contains less than 20% of the reporting level for all target compounds. The analytical system must also be free of contaminates that interfere with the analysis of the target compounds.

12.1.6 The laboratory must fortify all samples with surrogate standard solutions and calculate the percent recovery of each surrogate compound.

12.1.7 The laboratory must analyze a duplicate matrix spike sample for every 20 samples. Include at least one set of matrix spikes in every sample batch.

13.0 CALIBRATION AND STANDARDIZATION

13.1 Initial Calibration:

13.1.1 Each instrument is calibrated according to the procedures specified within EPA Method 8260B and EPA Method 624. Clarification of the calibration requirements and practices of this laboratory are discussed below. Refer to the EPA method protocols for additional details.

13.1.2 Analytical standards for the initial calibration must be certified and NIST traceable. The standard solutions for the calibration and standard spiking solutions must be from independent sources. The term

"independent source" means that the origin of the standard preparations is known to be different from one another. In practical terms this requires that the solutions be prepared by two different suppliers or at a minimum, have different lot numbers from the same supplier.

13.1.3 Initial calibration of the GC/MS is performed when a new capillary column is installed, the CCC criteria of +/-20% cannot be met or the minimum response factor criteria for the SPCC's cannot be met.

13.1.4 Five standard concentration levels, with the lowest level equal to or below the regulatory needs of the samples you are analyzing, are used to create the initial calibration curve. All quantitation is carried out using the internal standard technique. Paper copies of the calibration and quantitation reports are stored in a file.

13.1.5 The purpose of the CCCs is to evaluate the calibration from the standpoint of the integrity of the system. The %RSD for the CCCs in the initial calibration must be equal or less than 15%. The CCCs are:

1,1-Dichloroethene Toluene Chloroform Ethylbenzene
1,2-Dichloropropane Vinyl chloride

13.1.6 The SPCC's are used to check compounds stability and to check for system degradation. The average response factor for each SPCC (System Performance Check Compound) are as follows:

Choromethane > 0.100
1,1-Dichloroethane > 0.100
Bromoform > 0.100
Chorobenzene > 0.300
1,1,2,2-Tetrachloroethane > 0.300

13.1.7 The retention time of the target analytes in each calibration standard must agree within ± 3.0 seconds.

13.1.8 A reference mass spectrum library is used for all the target compounds. The reference mass spectra are used for qualitative and quantitative analysis as described later in this section. Refer to the operator manual for each mass spectrometer for procedures on using the reference mass spectrum library.

14.0 PROCEDURE

14.1 Sample preparation

14.1.1 All glassware must be cleaned with Alconox or equivalent detergent, rinsed with hot tap water and rinsed with Methanol prior to use.

14.1.2 Determine which samples will be prepared and analyzed. Samples may be assigned by the Laboratory Manager or Technical Director.

14.1.3 The assigned samples must be removed from the refrigerators.

14.1.4 Each sample must be visually inspected and double checked with the login information before setting up the run

14.1.5 Prepare samples by checking the labels with your sample ID's and performing any required dilutions.

14.1.6 If a sample has been submitted as a duplicate and/or matrix spike it is treated as such at this time. If one has not, then the analyst must prepare one using laboratory reagent water. The matrix spike level is normally 50 µg/L, which is prepared by spiking 8.0 µL of the a 1:10 dilution of the matrix spike standard solution into each of the two sample duplicate vials. Mix well by gently rotating the vials on their side.

14.1.7 The internal standard and surrogate compounds are automatically added to each sample by the auto sampler. The auto sampler is equipped with a 1 µL loop that rotates into the sample path during sample introduction into the Purge & Trap concentrator. The 1 µL loop injects 250 ng of each internal standard and surrogate compound as a 5 mL aliquot of sample is transferred into the Purge and Trap sparger. The final internal standard and surrogate concentration in the sample aliquot is 50 µg/L.

14.2 Handling difficult samples:

14.2.1 Water samples with excessive solids or suspended particulates may be centrifuged and transferred to a zero headspace VOA vial. Water from more than one vial will be required to do this. Make a note explaining what was done in the VOC logbook.

14.2.2 Samples analyzed with headspace or an inverted septum must have a comment added in the job folder and VOC logbook.

14.2.3 Broken, missing or frozen sample vials must be reported to the Laboratory Director. Also, inform the Laboratory Director immediately if the vials in a sample container do not look similar. The customer must be notified, if possible, prior to sample analysis and informed of the irregularities. Add a comment to the job file for the affected samples.

14.3 Sample Analysis Procedure

14.3.1 Instrument Conditions - The instrument conditions are set to the following parameters.

Note: The GC/MS software used by the Perkin-Elmer instruments is designed to collect MS data, setup calibration files, automatically perform calculations, report pass/fail for tuning, and report detect/non-detect for compound identification based on the method criteria. The analysts however should always review carefully data generated by the instruments for accuracy.

14.3.1.1 GC Parameters:

14.3.1.1.1 Carrier Control: Flow-He

14.3.1.1.2 Column Length: 30.00 meters, Restek #RTX-VMS 0.25mmID-1.40 umdf

14.3.1.1.3 Cat#: 19915, Serial #450192.

14.3.1.1.4 Vacuum Compensation: On

14.3.1.1.5 Split Flow: 50.0 mL/min.

14.3.1.1.6 Initial Setpoint: 1.00 mL/min.

14.3.1.1.7 Initial Temp: 45 C

14.3.1.1.8 Initial Hold: 2.00 min.
14.3.1.1.9 Ramp 1: 6.0/min to 150, hold for 0.00min
14.3.1.1.10 Ramp 2: 8.0/min to 200, hold for 0.00min
14.3.1.1.11 Total Run Time: 25.75 min.
14.3.1.2 Mass Spec Parameters:
14.3.1.2.1 Type: MS Scan
14.3.1.2.2 Ion Mode: El+
14.3.1.2.3 Data Format: Centroid
14.3.1.2.4 Start Mass: 35.00
14.3.1.2.5 End Mass: 300.00
14.3.1.2.6 Scan Time(sec): 0.20
14.3.1.2.7 Interssan Time(sec): 0.10
14.3.1.2.8 Start Time: (min): 0.96
14.3.1.2.9 End Time: (min): 25.00

14.3.1.3 GC/MS Tuning: The GC/MS system must be checked to ensure that acceptable performance criteria are achieved for the tuning compound Bromofluorobenzene (BFB) at the beginning of each day and every 12 hours thereafter for as long as analysis are to be performed. This performance test must be passed and maintained before any samples or standards are analyzed.

14.3.1.3.1 The internal standard and surrogate solution, which is added automatically by the auto sampler, contains BFB as one of the surrogates. Analysis of laboratory reagent water blank will serve as a GC/MS performance test.

14.3.1.3.2 The tune analysis must meet the criteria listed in EPA Methods 624 and 8260B for a 250-ng injection of BFB (bromofluorobenzene). Obtain the BFB mass spectrum and confirm that all of the m/z criteria in Chart 1 are achieved. If the criteria are not achieved, the operator must retune the mass spectrometer and repeat the test until all criteria are achieved.

Table1.-BFB m/z Abundance Criteria

Mass, m/z Abundance Criteria
50 15-40 % of mass 95
75 30-60 % of mass 95
95 Base peak, 100 %
96 5-9 % of mass 95
173 <2 % of mass 174
174 >50 % of mass 95
175 5-9 % of mass 174
176 >95 % but < 101 % of mass 174
177 5-9 % of mass 176

14.3.2 Sample Analysis Sequence The analysis sequence shall be the following:

14.3.2.1 BFB tune check.

14.3.2.2 Laboratory water blank (the tune and blank checks can be performed together).

14.3.2.3 50ug/L calibration check standard CCC at the beginning, after every 10 samples and at the end of each run.

14.3.2.4 50ug/L system performance check SPCC every 12 hours.

14.3.2.5 50ug/L Laboratory Control Sample (LCS).

14.3.2.6 Laboratory reagent blank.

14.3.2.7 Samples (including duplicate samples) up to eight.

14.3.2.8 Matrix spike sample and Matrix Spike Duplicate sample.

14.3.2.9 50ug/L continuing calibration verification.

14.3.3 Pre-analysis Checklist for the GC/MS run sequence:

14.3.3.1 Check the GC temperature program.

14.3.3.2 Check the auto sampler program.

14.3.3.3 Check the quantity of internal standard.

14.3.3.4 Empty the auto sampler waste bottle.

14.3.3.5 Fill the auto sampler wash bottle with laboratory reagent water and pressurize it.

14.3.3.6 Check the purge and trap parameter settings.

14.3.3.7 Check the job folders and field sheets for any special instructions.

14.3.3.8 Setup the acquisition sequence in the VOC logbook and in the computer acquisition program.

14.3.3.9 Check the acquisition parameters in the computer program.

14.3.3.10 Arrange the sample vials in the auto sampler tray.

14.3.3.11 Double check the order of the sample vials to insure that it is consistent with the computer acquisition and the VOC logbook.

14.3.3.12 Check the volume of internal standard/surrogate standard vials on the OI Analytical 4552 auto sampler.

14.3.4 Post-analysis Checklist: The results of each analytical run must be examined promptly upon completion. The instruments are designed to create both an electronic file and hardcopy for each sample analyzed. The following items must be checked following each batch analysis:

14.3.4.1 Check all tune blanks for compliance with tuning requirements.

14.3.4.2 Check to make sure the internal standards and spikes are in the retention time window and their mass spectra are correct.

14.3.4.3 Check the surrogate and spike recoveries against acceptance limits. As a guide, the recovery limits for the surrogates

are approximately 80 to 120%. The recovery limits for the spike compounds are approximately 70 to 130%.
14.3.4.4 Inspect the vials on the auto sampler tray to insure each was properly sampled.
14.3.4.5 The laboratory reagent water blanks must be free of target compounds.
14.3.4.6 Duplicate sample results must be comparable. Reanalyze the sample if the duplicate varies by more than 20%.
14.3.4.7 All detected target compounds must be within the calibration range. Reanalyze samples at a more appropriate dilution level if compounds are out of range.
14.3.4.8 Hardcopies of all sample data must be put into the associated job folders.
14.3.4.9 Hardcopies of the blanks, QC samples, calibration samples, matrix spike must be kept with the data.

15.0 CALCULATIONS

15.1 Quantitative analysis

15.1.1 The quantitation of identified compounds is based on the integrated abundance of the extracted ion current profile of the primary characteristic ion(s). The internal standard used for quantitation is the one nearest to the retention time of the analyte.
15.1.2 The average response factors from the initial calibration are used to calculate the concentration of each compound in the sample. Amounts are calculated as follows: Amount = Peak Response/Average RF
Where: Peak Response = Area of the peak calculated * Amount of ISTD
Area of ISTD
Where: Average RF is calculated internally by the Perkin Elmer software at calibration by taking the least squares fit of the calibration line and then taking the best slope of that line. ISTD is the internal standard.

16.0 METHOD PERFORMANCE

See Validation Report.

17.0 POLLUTION PREVENTION

17.1 All chemicals and standards are to be used under a fume hood and disposed of into appropriate waste containers.
17.2 All water samples are to be neutralized before they are disposed of down the sink.
17.3 All samples that are deemed hazardous are returned to the client for disposal.

18.0 DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QC MEASURES

18.1. Internal Standard Retention Time and Internal Standard Response:

The internal standard responses and retention times of each standard and sample analyzed are evaluated after data acquisition. If the retention time for any internal standard changes by more than 3.0 seconds from the most recent initial calibration, then the chromatographic system must be inspected for malfunctions and corrections must be made, as required. If the extracted ion response for any internal standard varies by more than 50% from the last initial calibration, the GC/MS, purge and trap and auto sampler system must be inspected for malfunctions and corrections must be made, as appropriate. Any standard or sample failing these internal standard checks are re-analyzed. The system is re-calibrated, if necessary.

18.2 Qualitative analysis:

Qualitative identification of each compound is based on retention time and comparison of the sample mass spectrum with characteristic ions in the reference mass spectrum. The characteristic ions from the reference mass spectrum are defined to be the three ions of greatest intensity or any ions over 30% relative intensity. Compounds are identified as present when the following criteria are met:

18.2.1. The characteristic ions of a compound maximize in the same scan or within one scan of each other.

18.2.2. The retention time of the compound is within ± 3.0 seconds of the retention time of the standard component.

18.2.3. The relative intensities of the characteristic ions agree within 20% of the relative intensities of these ions in the reference spectrum. The presence of co eluting compounds may alter the relative intensities and complicate the compound identification. Examination of extracted ion current profiles against the library can aid in the selection of spectra and in qualitative identification of compounds.

18.2.4. Structural isomers that produce similar mass spectra and are sufficiently resolved are identified as individual isomers. If the height of the valley between two close eluting isomers is more than 25% of the sum of the two peak heights, then the structural isomers must be identified as isomeric pairs.

18.2.5. A library search must be performed for unknown chromatographic peaks when the total ion chromatogram of the sample contains unknown peaks larger than the internal standard peaks. A tentative identification can be assigned provided that relative intensities of major ions in the library reference spectrum are present in the sample spectrum and the relative intensities agree within ±20%. Molecular ions must also be present.

18.3. Quantitative analysis:

18.3.1. The quantitation of identified compounds is based on the integrated abundance of the extracted ion current profile of the primary characteristic ion(s). The internal standard used for quantitation is the one nearest to the retention time of the analyte.

18.3.2. The average response factors from the initial calibration are used to

calculate the concentration of each compound in the sample. Amounts are calculated as follows: Amount = Peak Response/Average RF
Where: Peak Response = Area of the peak calculated * Amount of ISTD
Area of ISTD
Where: Average RF is calculated internally by the Perkin Elmer software at calibration by taking the least squares fit of the calibration line and then taking the best slope of that line.
ISTD is the internal standard.

18.4. Data Archival

18.4.1. Perkin Elmer Turbo mass Gold files:

18.4.1.1. Three different types of files are generated by the Perkin Elmer instruments: a raw data file with extension .RAW, sample list file with extension .SPL and calibration files with extension. CAL.

18.4.1.2. All data files generated from the Perkin Elmer instrument are kept in the C: directory under VOCMS.PRO and categorized by date.

18.4.1.3. Once the data is reviewed and final reports are generated, the data files on the instrument are permanently archived on optical disk by the Laboratory Manager on a weekly basis.

18.4.1.4. Contact the Quality Control Officer if raw data files need to be retrieved from archival and identify the files to be retrieved.

18.5. Data Processing

18.5.1. Turbo mass Gold Automated Quantification follows six basic steps.

18.5.1.1. Creation of a list of samples using the Sample List Editor. This is a list of samples for analysis.

18.5.1.2. Acquisition of each sample in the analysis. .

18.5.1.3. Integration of data file chromatograms. This is done by the following tasks: Integration of a chromatogram trace to obtain peak information. Location of the chromatogram peak relating to a specific compound from the list of detected peaks. Calculation of a response factor for the located peak and the formation of a Quantifiable calibration curve.

18.5.1.4. Run Assignments: A run is a group of samples including precision and spike recovery samples and all of the associated samples analyzed together by the same method. Run ID's are determined by the Laboratory Manager.

18.5.1.5. Spike Code: Assign the appropriate spike code for the spikes by using the following: MS for Matrix Spike, MSD for Matrix Spike Duplicate, BLSK for Blank Spike.

18.6.2. Results Calculation

18.6.2.1. Before calculation, samples are selected from the Sample List screen. Process the samples by choosing the Process option from the Quantify menu.
18.6.2.2. When the calculations are finished, select View Results from the Quantify menu. The results are displayed in three windows: the Graphs window, the Summary window and the Peak List window.

18.7. Data Review

18.7.1. Ensure that method blanks, reagent blanks, matrix spikes, duplicate matrix spikes, duplicate samples, continuing calibration standards and system monitoring check standards are analyzed at the frequency specified below:

Type of Check	**Frequency Performed**
Method Blanks	≥ 10% of samples
Matrix Spikes	≥ 5% of samples
Duplicate Samples	≥ 5% of samples
CCC	Initial and every 10 samples and at the end of the run.
SPCC	Initial and every 12 hours

18.7.2. Blanks must be free of target compounds and interferences. The level of target compounds in the blanks must be less than 20% of the reporting limit. If the reporting limit and the quantitation limit are the same and target analytes were present in the blank above the reporting limit but not in the samples and spiked samples were within control limits, then the samples can be reported. If target analytes are present in the blank and in the samples above the reporting limit, the samples shall either be rerun or the data flagged if samples are outside the holding time. The flag shall be a note in the case narrative addressing the target analyte found and the subsequent target analyte found in the blank. There are several types of blanks including: Method, Extraction and Trip.

Type of Blank	**Frequency Performed**
Method	≥ 10% of samples
Extraction	Every extraction batch
Trip	Every job request

18.7.3. Examine QC data of the CCC and SPC check. The QC code ‘P/F’ will indicate whether the result is in or out of control limits. Report persistent or unusual problems to the Technical Director so corrective action can be taken.
18.7.4. Recovery and Precision (RPD) statistics are determined for samples fortified with known quantities of target analytes. The recovery is an indication of the accuracy of the analytical result. The RPD (relative

percent difference) from duplicate spiked samples is an indication of precision. Examine the recovery and precision data to ensure that they are within control limits for the requested analytes. If the recovery limits or precision measurements are not within laboratory control limits, the affected samples should be reanalyzed if possible. Results may be qualified if the 2nd results fail or the samples are expired.

18.8 Data Reporting by Analyst

18.8.1. Select component results for reporting.

18.8.2. Add appropriate comments whenever results are qualified or comments are otherwise warranted. Indicate all actions taken, such as reanalysis or dilutions, required to resolve a detected problem.

18.8.3. Prepare Job Folders: A job folder contains all data associated with the analysis of samples within a given request. Thus, a folder may contain data acquired on different days, different instruments or different tests. Every effort must be taken to keep the data complete and orderly.

• Make copies of the field sheets for each job folder
• Put hardcopies from the sample screening in the job folders
• Hardcopies of all sample data must be put into the associated job folders.
• Hardcopies of the blanks, QC samples, calibration samples, matrix spike must be put with the data.
• Sign the files to indicate initial data review is complete. Put a copy of the analysis sequence into each job folder.
• Check the job folder a final time for special directions and completeness.
• Double check calculations and final reported values.
• Give the complete job folder to the Laboratory Manager for data review and authorization.

18.9. Data Reporting by Technical Director:

• Review each job folder according to the criteria found in **Section 18.7**.
• Make note of any necessary corrections and return to the analyst. The analyst must make all corrections and review a second time before resubmitting.
• Return the job folders to the analyst for archival.
• Report to the Technical Director that the job is complete and ready for final review.

19.0 CORRECTIVE ACTION FOR OUT-OF-CONTROL DATA

19.1 All corrective action will be administered by the Laboratory Director and or the Technical Director.

19.1.1 The Laboratory Director and or Technical Director will identify and determine the out of control data in the data review process.

19.1.2 The data will be flagged on the Quality Assurance Summary and noted in the case narrative of the final report to the client by the Technical Director.

19.1.3 An internal out of control report will be filled out by the Laboratory Director and or the Technical Director.

19.1.4 Supervisors and Analysts that were involved in the out of control data generation will be interviewed to determine possible causes for the situation.
19.1.5 Refer to SOP admin-014 for details on corrective action.

20.0 CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 The Laboratory Director and or Technical Director will handle unacceptable data in the following manner:

20.1.1 If possible the sample or samples will be re-extracted and or re-analyzed.
20.1.2 If re-extraction and or re-analysis is not possible the data will be flagged by the Technical Director in the Quality Assurance Summary and the Case Narrative report to the client.
20.1.3 If the data has already been reported to the client, the client will be contacted by the Technical Director and informed of the situation. The client will be given the option of accepting the data as is or re-sampling.

21.0 WASTE MANAGEMENT

21.1. Store any wastes that cannot be disposed of down the in 2 L bottles (properly labeled with **Hazardous Wastes** labels).
21.2. All the non-hazardous wastewater can be dumped into sink if its pH range is 5-10. If it is not, the wastewater has to be neutralized before dumping.

22.0 REFERENCES

22.1. *Code of Federal Regulations, Title 40, Part 136, Vol. 49, No. 209*, Method 624: Base/Neutrals and Acids, 10/26/84.
22.2. *Test Methods for Evaluating Solid Wastes, Fourth Edition, SW-846,* Method 8260B, Revision 2, 12/96.
22.3. *Test Methods for Evaluating Solid Wastes, Fourth Edition, SW-846,* Method 8000, Revision 2, 12/96.
22.4. PerkinElmerTurbomass Software Manual, Manual Number: 09934468, Manual Date: May 2001.
22.5. PerkinElmerTurbomass Gold GC/MS Hardware Manual, Manual Number: 09934469, Manual Date: May 2001.
22.6. OI Analytical Model 4560 Sample Concentrator Operator's Manual , Document Part Number 227959, Revision 2.2, August 1999.
22.7. OI Analytical Model 4552 W/S Autosampler Operator's Manual , Document Part Number 285007, Revision 2.3, November 1999.
22.8. Comprehensive Quality Assurance Plan, Chemistry Section, Bureau of Labs, DEP, April 1999.
22.9. FDEP Laboratory Safety SOP Manual.