California Environmental Protection Agency

# Air Resources Board

**Northern Laboratory Branch**
**Monitoring and Laboratory Division**

# STANDARD OPERATING PROCEDURE FOR THE ANALYSIS OF LEVOGLUCOSAN, MANNOSAN, AND GALACTOSAN IN AMBIENT AIR USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY

**SOP MLD073**
Version 1.0
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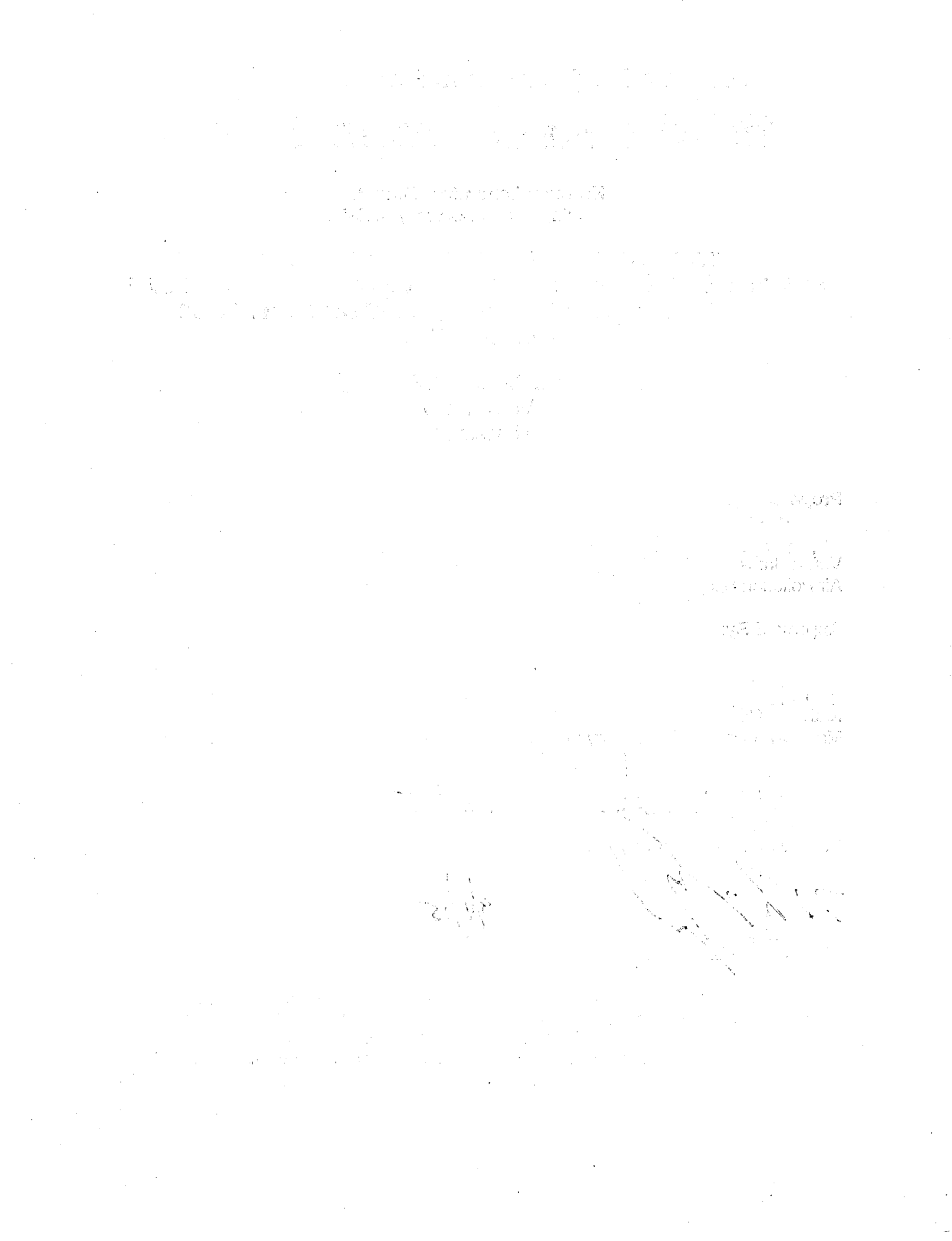

# SOP MLD073

## STANDARD OPERATING PROCEDURE FOR THE ANALYSIS OF LEVOGLUCOSAN, MANNOSAN, AND GALACTOSAN IN AMBIENT AIR SAMPLES USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY

## 1.0 SCOPE

This document describes a method for the analysis of levoglucosan (1,6-anhydro-β-D-glucopyranose), mannosan (1,6-anhydro-β-D-mannopyranose), and galactosan (1,6-anhydro-β-D-galactopyranose) in ambient air samples. The analysis of these compounds is important for understanding the impact of wood smoke on air quality.

## 2.0 SUMMARY OF METHOD

Particulates in ambient air samples are collected on $PM_{2.5}$ Teflon filters from ARB's $PM_{2.5}$ Speciation network. The network employs the Met One Spiral Aerosol Speciation Samplers (SASS). Each sampler is programmed to pump approximately 9.7 $m^3$ total volume of ambient air through a Teflon filter in a 24-hour time frame.

Method MLD073 determines the concentrations of levoglucosan, mannosan and galactosan collected on the $PM_{2.5}$ Teflon filters. The Teflon filters are cropped out of the plastic holding rings and then extracted with carbonyl-free acetonitrile by means of ultrasonication. The sample extracts are filtered through 0.2 µm Teflon filters. A silanizing reagent is added to an aliquot of sample extract to form silyl ethers of the compounds. The derivatized extract is analyzed by a gas chromatograph (GC) coupled with a mass spectrometer (MS). Compounds are identified by both retention time and mass fragments. The response of primary quantitation ions is used for measurement of the target compounds in the extract.

## 3.0 EQUIPMENT AND SUPPLIES

3.1 Gas Chromatograph: system with programmable oven, electronic pressure control for capillary columns, heated injector, and automated liquid injector

3.2 Column: Agilent VF-5 ms, 30 m x 0.25 mm (0.25 µm) or equivalent

3.3 MS Detector: capable of scanning the mass range from 100 m/z (mass to charge ratio) to 400 m/z

3.4 Filters: $PM_{2.5}$ Teflon (PTFE) filters such as those manufactured by Measurement Technology Laboratories, LLC (Item #: PT47-EP) measuring 46.2 mm in diameter. Each filter is supported with a plastic ring

3.5 4 mL storage vials with Teflon lined screw caps such as VWR part# 66009-876

3.6 GC/MS autosampler vials with inserts such as Agilent part# 5182-0715 with 250 µL inserts (part# 5181-3377)

3.7 Ultrasonic bath: temperature programmable such as Branson model 8510

3.8 Water bath: capable of maintaining a temperature of 65 to 70 degrees centigrade

3.9 15 mL extraction tubes, such as VWR polypropylene centrifuge tubes with plug seal caps, item 21008-103

3.10 Syringe and syringe filters such as Exel disposable syringes, product # 550-30577, and VWR 0.2 µm Teflon disposable syringe filters, item# 28145-491

3.11 Volumetric flasks: 5, 25, 50 mL volumes

3.12 Analytical balance

3.13 Eppendorf manual and electronic hand dispensers with disposable pipette tips: 10-100, 20-300, 50-1000, 100-5000 µL volume ranges

3.14 Disposable polyethylene transfer pipettes, 1.5 mL, such as VWR item # 16001-192

3.15 Forceps, stainless steel, such as VWR item 25716-002

3.16 Scissors, stainless steel, such as Spectrum Chemical product # 142-11484

3.17 Disposable nitrile gloves used to handle organic solvents

3.18 Hamilton microliter syringes (or equivalent): 10 µL, 25 µL, 250 µL volumes

## 4.0 REAGENTS

4.1 Carbonyl-free acetonitrile, CAS No. 75-05-8, such as Burdick & Jackson catalog # 018-4

4.2 Levoglucosan, 1,6-Anhydro-β-D-glucopyranose, CAS No. 498-07-7

4.3 Mannosan, 1,6-Anhydro-β-D-mannopyranose, CAS No. 14168-65-1

4.4 Galactosan, 1,6-Anyhdro-β-D-galactopyranose, CAS No. 644-76-8

4.5 Mixed silanizing reagent (BSA + TMCS + TMSI 3:2:3), Supelco item 3-3151, 0.1 mL/ampule

4.6 Helium ultra-high pure (UHP), 99.999% for use as the GC column carrier gas

4.7 Perfluorotributylamine (FC43) for use in MS tuning

## 5.0 SAFETY

The analyst must wear protective eyewear, lab coat, and nitrile gloves whenever working with standards, solvents, silanizing agents, and solutions and when handling extracts. Silanizing agents are flammable and corrosive, other reagents used are skin and eye irritants. Refer to safety data sheets for specifics.

This method uses high pressure gases. Refer to the safe handling practices regarding compressed gases when moving and installing the cylinders.

The GC and MS have heated zones which may cause burns. Avoid contact with these zones and devices when in operation and make certain they are de-energized or at ambient temperature prior to servicing.

Waste disposal must be followed in accordance with the Chemical Hygiene Plan.

## 6.0 STANDARDS PREPARATION

All standard solutions are stored in a refrigerator at 4°C until used. The standard solutions are removed from the refrigerator and allowed to equilibrate to room temperature before use. The 100 µg/mL composite standard solutions are stable for 11 months when stored properly.

### 6.1 Calibration, Check, and Spike Standards

6.1.1 Individual stock standard solutions are prepared by dissolving a reagent grade solid compound (galactosan, mannosan, or levoglucosan) in carbonyl-free acetonitrile. To make an approximate 1000 µg/mL solution, weigh 25 mg into a 25 mL volumetric flask and bring to volume with carbonyl-free acetonitrile. Ultrasonication of the solution may be necessary to completely dissolve the compound.

6.1.2 A composite standard is prepared by combining 5mL of each individual stock standard solution in a 50 mL volumetric flask and bringing to volume with carbonyl-free acetonitrile. The concentration of this composite standard will be 100 µg/mL each for galactosan, mannosan, and levoglucosan.

6.1.3 Calibration and check standards: The following table lists the dilutions used to prepare calibration standards. These standards are made by combining each required amount of composite standard diluted with carbonyl-free acetonitrile. They are prepared in GC autosampler vials and are used as calibration standards in an analytical sequence. A subset of the calibration standards (one or more) are injected again at the end of the analytical sequence as check standards. These standards may be used for up to two weeks after preparation.

For example, to prepare a Calibration Level 1 standard, combine 995 µL of carbonyl-free acetonitrile and 5 µL of the 100 µg/mL composite standard in a GC autosampler vial. The final concentration of this standard will be 0.5 µg/mL.

| Calibration Level | Composite Standard Conc. (µg/mL) | Volume of Composite Standard Used (µL) | Final Volume (mL) | Working Standard Conc. (µg/mL) |
|---|---|---|---|---|
| 1 | 100 | 5 | 1.0 | 0.5 |
| 2 | 100 | 10 | 1.0 | 1 |
| 3 | 100 | 20 | 1.0 | 2 |
| 4 | 100 | 40 | 1.0 | 4 |
| 5 | 100 | 60 | 1.0 | 6 |
| 6 | 100 | 80 | 1.0 | 8 |
| 7 | 100 | 100 | 1.0 | 10 |

6.1.4 Spike Standards: The 100 µg/mL composite standard is also used as a spike standard as described in section 7.0.

6.2 Control Standard

Control stock standards are prepared as described in section 6.1.1, using reagent grade solid compounds obtained from a second source. The control stock standards are used to prepare a 100 µg/mL composite control standard, as described in section 6.1.2.

A mixed working control standard is prepared from the composite control standard by combining 950 µL of carbonyl-free acetonitrile and 50 µL of the 100 µg/mL composite control standard in a GC autosampler vial. The final concentration of this standard will be 5 µg/mL of each compound. This standard is used as the control standard in an analytical sequence.

## 7.0 EXTRACTION AND DERIVATIZATION

Samples collected on Teflon filters are stored in a refrigerator at 4°C until extraction. Prior to extraction, the Teflon portion of the $PM_{2.5}$ filter sample is removed from the plastic ring using scissors assisted with forceps. The plastic ring is discarded, and the filter is placed in a 15 mL centrifuge tube. 2 mL of carbonyl-free acetonitrile is added, and the tube is securely capped. The centrifuge tube is placed in an ultrasonication bath for 60 minutes while tap water in the bath is held at 40°C. After sonication, the extract is filtered with a syringe coupled with a 0.2 µm Teflon syringe filter into a 4 mL sample storage vial.

For each batch of 10 samples, one unexposed $PM_{2.5}$ filter is extracted and used as a filter blank. This blank is reported as the Method Blank.

For each batch of ten samples, a laboratory control spike sample is prepared by pipetting 40 µL of the 100 µg/mL composite standard (Section 6.1.4) onto an unexposed $PM_{2.5}$ filter before extracting it using the same procedures used on samples. The expected final spiked concentrations of levoglucosan, mannosan, and galactosan are equivalent to the 2 µg/mL standard.

Sample extracts are stored in a refrigerator at 4°C until derivatization. Sample extracts remain stable for 60 days when kept refrigerated.

Due to the polar nature of the target compounds they cannot be analyzed directly and must be derivatized. Prior to analysis, a 100 µL aliquot of each extracted sample, spike, or blank is placed in a 250 µL vial insert contained in an autosampler vial. The Teflon lined cap is secured on the vial. 20 µL of silanizing reagent is added via syringe to the 100 uL aliquot through the septa in the vial cap. The vial is placed in a water bath at 70°C for 60 minutes. The vial is removed, dried, and placed in the GC autosampler. GC analysis of the sample set must begin immediately after derivatization.

Standards are made by taking 100 µL aliquots of working standards (6.1.3.) prepared in acetonitrile. The standards are then derivatized with the samples.

## 8.0 ANALYSIS

### 8.1 Instrument Performance Check

The MS must be tuned with FC43 using the manufacturers automated tuning program. Tuning is not required prior to every analytical set; however it must be done at least every two months and whenever maintenance has been performed on the ion trap or when instrument problems are suspected.

The tune values, with regard to positions and abundance ratios of the tune m/z's and their corresponding isotope m/z's, must be reviewed. The system leak and electron multiplier voltage are also checked and evaluated. The tuning report is saved in the data folder or tune folder and is referenced by date of tune. If any discrepancies or abnormalities are noted the tune is to be rerun. If problems with the tune results are duplicated a corrective action must be undertaken before analyzing samples.

8.2 GC/MS Initial Setup

Typical gas chromatograph, mass spectrometer settings and programs may be found in Appendix MLD073 A1. .

8.3 Injection Scheme

Each analytical run of 10 or fewer samples must include bracketing standards, controls, and blanks (see section 9.0 for descriptions of blanks) as listed below. The recommended order of analysis is as follows:

- Solvent Blank (carbonyl-free acetonitrile)
- Multipoint Calibration Standards (three point minimum)
- Control Standard
- Solvent Blank
- Filter Blank (Method Blank)
- Samples (no more than 10)
- Sample Duplicate (usually first sample in set is reanalyzed)
- Laboratory Control Spike
- Check Standard(s)
- Any other lab samples such as dilutions
- Check Standard(s)

A typical analytical run is shown in Appendix MLD073 A2.

## 9.0 QUALITY CONTROL

9.1 Blanks

9.1.1 A method blank must be analyzed before any sample is run. The method blank is an unexposed $PM_{2.5}$ Teflon filter that is taken through the extraction/derivatization process with its corresponding set of samples to demonstrate that no contaminants were introduced during extraction. The result of any single analyte in the blank must not exceed the limit of quantitation (LOQ) in order to validate any subsequently analyzed samples. One method blank is included with ten air samples.

9.1.2 A solvent blank is a vial of carbonyl-free acetonitrile. It has not been exposed to a blank $PM_{2.5}$ Teflon filter, nor taken through the derivatization process. Solvent blanks are analyzed, at a minimum, at the beginning of a sample set to demonstrate that the

analytical system is free from interferences. Additional solvent blanks may be analyzed elsewhere in the sample set at the bench chemist's discretion, if needed (i.e., after an anticipated high concentration sample to prevent contamination of subsequent samples.)

9.1.3 A field blank is an unexposed $PM_{2.5}$ Teflon filter shipped to the laboratory by sampling personnel. Field blanks are taken through the extraction and derivatization process, and are analyzed and reported as samples. Field blanks are recommended to be collected and analyzed on a quarterly basis or as requested by the laboratory.

9.2 Laboratory Control Spikes

Method accuracy is measured through spiked samples. Spikes are prepared by adding spike standards to unexposed filters as described in section 7.0. A spiked filter is extracted and analyzed with each set of ten ambient air samples. The spike recoveries are calculated as:

$$\% \text{ spike recovery} = \frac{(M1 - M2)}{C} \times 100$$

M1 = Measured concentration of spiked blank filter

M2 = measured concentration of filter blank

C = Expected analyte concentration of spike

For a valid spike analysis, the percent spike recovery should be between 70 to 130 percent. If the percent recovery is outside of this range, the problem must be identified and corrected. All associated samples in the batch must be reanalyzed after correcting the problem.

9.3 Multipoint Calibration

A multipoint calibration analysis must be performed to determine instrument precision and linearity of the GC/MS response for the target compounds. This is done by performing analyses of a minimum of three concentration levels of the component standard mix. Multipoint calibrations may range in concentration from 0.5 µg/mL to 10 µg/mL. A multipoint analysis must be performed with each sample set prior to analysis of samples. In order for the calibration curve to be linear, the correlation coefficient (r) of a linear regression analysis must be greater than or equal to 0.98.

A minimum of one check standard is analyzed at the end of every sample set to verify the stability of the instrument calibration. The check standard is a midpoint of the calibration standard. A control standard analyzed at the end of the sample set may serve as a check standard. Any bracketing standards analyzed in the sample set may also serve as a check standard if needed. Results must be within 30 percent of the expected value. If results exceed this criterion, the cause must be investigated and the sample set must be reanalyzed.

9.4 Limit of Detection (LOD) Determination

The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix (including sampling media) containing the analyte. The LOD is equivalent to the MDL. The procedure used to determine the LOD is documented in NLB's Laboratory Quality Control Manual and 40CFR Part 136 Appendix B. The LODs as calculated by this multiple replicate method are presented in Appendix MLD073 A4.

9.5 Limit of Quantitation (LOQ)

The lower level where measurements become quantitatively meaningful is called the limit of quantitation and is defined as:

$$LOQ = 10 \times s$$

Where s is the standard deviation of at least seven replicate analyses of the lowest calibration standard.

The lowest standard should typically be at or below the LOQ to verify the system has sufficient sensitivity to confidently report values at LOQ. Results are not reported below the method LOQ.

9.6 Control Standard

The control standard is analyzed with every analytical set to evaluate the accuracy of calibration and the overall system performance. Analysis results of the target compounds are recorded and used to generate control charts. Typically 20 data points are needed to initially establish control limits. The average and standard deviation for the 20 control data points for each analyte are then determined. If percent relative standard deviations (RSD) for these data points are less than five percent, the percent RSD is adjusted upward to five percent. The percent RSD or its

adjusted value is used to determine control limits. The average concentration of the 20 data points plus and minus three times the percent RSD define the upper and lower control limits, respectively. Virtually all of the measured concentrations produced by a system that is in control will fall between these limits. Warning limits are defined as the average concentration plus and minus two times the percent RSD. Ninety-five percent of the measured concentrations will fall within the warning limits when the system is in control.

Each analytical set's control value is compared to the current control chart to establish that the method is in statistical control. Control standard analysis results must be within the pre-established control limits for sample data to be valid. One control standard is analyzed with each set of ten ambient air samples. If a control value is outside of the established control limits, the analysis is discontinued and the cause of the problem is investigated. All associated samples in the batch must be reanalyzed after correcting the problem. If a run of seven consecutive control values trends either upward or downward, new control standards must be prepared and new control limits established.

9.7 Duplicates

Duplicate samples are analyzed with each sample set of ten air samples. Precision is measured by the percent difference (% D) of the sample or standard duplicate analyses. Maximum allowable % D for the duplicate sample analyses are +/- 30 percent for each analyte.

$$\%\ D = \frac{|X1 - X2|}{\text{Average}} \times 100$$

Where:
X1 = first measurement value
X2 = second measurement value
Average = average of X1 and X2

If the % D is outside of +/- 30 percent, the analysis is discontinued and the cause of the problem is investigated. All associated samples must be reanalyzed after correcting the problem.

9.8 Dilutions

Dilutions are required when any sample concentration exceeds the calibrated linear range by more than ten percent. Dilution results are valid if the concentration falls within the calibrated linear range. Multiple dilutions are sometimes necessary.

## 10.0 INTERFERENCES AND LIMITATIONS

10.1 Interferences may be caused by contaminants in the filters, solvents, sample extraction apparatus, filtration apparatus, and glassware. A filter blank is extracted and analyzed with each set of samples to monitor these possible sources of contamination.

10.2 A laboratory control spike is prepared by depositing known amount of target standards onto a blank filter followed by the same processes of extraction and filtration as performed on the samples. One laboratory control spike is prepared and analyzed with every ten samples. Laboratory control spikes can suffer from background interferences inherent in the filter and cause lower recoveries of target compounds.

10.3 The MS should be setup and tuned according to the manufacturer's specifications prior to sample analysis.

10.4 Although the retention time of an analyte is not the only parameter used in identifying a component in GC/MS, the retention times of the GC portion of the system must meet QA manual requirements.

10.5 All target compounds are identified by their mass fragment fingerprint and retention times. Compounds having similar GC retention times may co-elute. This can lead to misidentification or inaccurate quantitation. The use of a proper compound specific primary quantitation ion, as well as secondary ions, allows accurate quantitation and identification even under these circumstances. There is no substitute for good chromatographic separation. Although this method uses micro-SiS (Selective ion Scanning) the ions selected comprise over 80 percent of the ion fragments for all three compounds which yields good identification spectra.

10.6 Very low target and non-target analyte concentrations may not produce a good fragment ratio match. This may result in either low match quality or misidentification and therefore should be evaluated by an experienced GC/MS operator.

10.7 Analysis of an analytical set must begin immediately following derivatization. Derivatized compounds' stability may become questionable after 24 hours because of water and competing reactions, resulting in low bias.

10.8 The analytical system may be contaminated when samples containing high compound concentrations are analyzed. If there is suspected carryover from a high concentration sample, the succeeding sample should be reanalyzed.

10.9 High boiling compounds being trapped on the column may cause baseline shifting, or the appearance of broad, extraneous "ghost" peaks. The column should be baked out to remove these contaminants prior to analyzing samples. The bake out temperature must not exceed the column's maximum operating temperature.

10.10 Historical data has shown that samples collected from May 1 – September 30 rarely have any positive results. Because of this, samples collected during this time frame are analyzed only on request. They are stored at 4°C for one year and then moved to a box and stored at room temperature. After two years, the samples are disposed.

## 11.0 CACLULATIONS

The concentrations of analyzed samples are initially reported in µg/mL.

Ambient air concentrations are reported as $\mu g/m^3$ and are calculated as:

$$\mu g/m^3 = \frac{\text{Analyzed Value (µg/mL) x Extract Volume (mL)}}{\text{Air Volume } (m^3)}$$

Ambient air concentrations may be converted from $\mu g/m^3$ to ppbv as follows:

$$ppbV = \frac{\text{Concentration } (\mu g/m^{3)} \times 24.46}{\text{molecular weight of target compound}}$$

Analyzed values will not be reported if below the LOQ.

## 12.0 DATA HANDLING

12.1 After data acquisition, the raw data files collected are processed by the analytical software to produce result files. The resultant files contain

quantitation information such as peak areas and retention times, along with mass spectral and instrumentation information.

12.2 Chromatographic peaks found in the total ion chromatogram (TIC) in the result files for calibration standards are qualitatively identified based on matching their mass fragments to reference fragments and their retention times to reference retention times. Both of these references are stored in the instrument method.

12.3 The instrument method is calibrated for both retention time and concentration during data processing using the integrated calibration standard areas for the primary quantitation ions. The concentrations of target compounds are based on the peak areas and the known analyte concentrations in the standards. Concentrations are calculated using the instrument standardization routine for samples, blanks, controls, and spikes.

12.4 All QC and sample results are verified by the chemist and then sent to the Laboratory Information Management System (LIMS) for archive and reporting. Data is reviewed by a peer chemist and management before being released. Data for the ambient toxics program are transferred from LIMS to the US EPA Air Quality System (AQS) database for public access.

## 13.0 MAINTENANCE AND REPAIR

Preventive maintenance is done on an annual basis on the GCMS and repairs are done as needed by an approved vendor under contract to MLD or by experienced staff. All maintenance and repairs are documented in a logbook.

## 14.0 REFERENCES

14.1 MLD QC Manual

14.2 NLB Chemical Hygiene Plan

## 15.0 APPENDIX

15.1 Appendix OLS MLD073 A1 GC/MS Instrument Method for MLD073

| | | |
|---|---|---|
| 15.2 | Appendix OLS MLD073 A2 | Typical Analytical Sequence for MLD073 |
| 15.3 | Appendix OLS MLD073 A3 | Target Compounds Validated by MLD073 |
| 15.4 | Appendix OLS MLD073 A4 | Replicate LOD Determination |
| 15.5 | Appendix OLS MLD073 A5 | Initial Control Values and Control Limits |
| 15.6 | Appendix OLS MLD073 A6 | Recommended Corrective Actions for QC Failures |
| 15.7 | Appendix OLS MLD073 A7 | Revision History |
| 15.8 | Appendix OLS MLD073 A8 | Review History |

## APPENDIX OLS MLD073 A1
## GC/MS Instrument Method for MLD073

### 8400 Autosampler

Syringe Size: 10 uL
Injection Mode: Std Split/Splitless
Solvent Penetration Depth: 90 %
Sample Penetration Depth: 90 %
Default Clean Vial: I
Default Clean Volume: 5.0 uL
Default Clean Strokes: 1
Default Clean Drawup Speed: 5.0 uL/sec
Clean Mode Pre-Inj Solvent Flushes: 3
Clean Mode Post-Inj Solvent Flushes: 6
Clean Mode Pre-Inj Sample Flushes: 0
Clean Mode Solvent Source: I

### 3800 GC

Middle Injector Type 1177
Oven Power: On
Temperature: 250 C

| Time (min.) | Split State | Split Ratio |
|---|---|---|
| Initial | Off | Off |
| 0.50 | On | 50 |
| 1.50 | Off | Off |

Middle Injector EFC Type 1
Constant Column Flow: 1.3 ml/min

### Column Oven

Stabilization Time: 0.10 min

| Temp (C) | Rate (C/min) | Hold (min) | Total (min) |
|---|---|---|---|
| 100 | 0.0 | 2.00 | 2.00 |
| 200 | 10.0 | 1.00 | 13.00 |
| 250 | 25.0 | 0.00 | 5.00 |

**4000 MS/GC**

Instrument Configuration: Internal EI
Mass Data Type: Centroid
Number Of Segments: 2
Method Start Time: 0.00 minutes
Segment: 1 Delay
Running Time: 0.00 - 9.00 minutes
Ionization: Off
Segment: 2 uSiS Scan
Running Time: 9.00 - 14.00 minutes
SetPoints:
Calibrant: Off
Scan Type: uSIS

| Precursor Ion (m/z) | Ionization Storage Level (m/z) | Isolation Window (m/z) | Low Offset (m/z) | High Offset (m/z) | High Mass Ejection (volts) |
|---|---|---|---|---|---|
| 204.0 | 39 | 3.0 | 0.0 | 0.0 | 35.0 |
| 217.0 | 39 | 3.0 | 0.0 | 0.0 | 35.0 |
| 333.0 | 39 | 3.0 | 0.0 | 0.0 | 35.0 |

Ionization Type: EI
Target TIC: 5000 counts
Max Ion Time: 25000 uSeconds
Emission Current: 20 uAmps
General Parameters:
Scan Speed: Normal
Scans Averaged: 3 microscans (1.21 seconds/scan)
Data Rate: 0.83 Hz
Mass Defect: 0 mmu/100u
Multiplier Offset: 0 volts
Count Threshold: 1

## APPENDIX OLS MLD073 A2
## Typical Analytical Sequence for MLD073

| Line # | Sample Type | Sample Name | Inj. | Vial # | Inj. Volume |
|---|---|---|---|---|---|
| 1 | Blank | Solvent Blank | 1 | 1 | 1.0 |
| 2 | Calibration | Standard 1 | 1 | 2 | 1.0 |
| 3 | Calibration | Standard 2 | 1 | 3 | 1.0 |
| 4 | Calibration | Standard 3 | 1 | 4 | 1.0 |
| 5 | Calibration | Standard 4 | 1 | 5 | 1.0 |
| 6 | Calibration | Standard 5 | 1 | 6 | 1.0 |
| 7 | Calibration | Standard 6 | 1 | 7 | 1.0 |
| 8 | Calibration | Standard 7 | 1 | 8 | 1.0 |
| 9 | QC | Control A | 1 | 9 | 1.0 |
| 11 | Blank | Method Blank | 1 | 11 | 1.0 |
| 12 | Sample | Sample #1 | 1 | 12 | 1.0 |
| 13 | Sample | Sample #2 | 1 | 13 | 1.0 |
| 14 | Sample | Sample #3 | 1 | 14 | 1.0 |
| 15 | Sample | Sample #4 | 1 | 15 | 1.0 |
| 16 | Sample | Sample #5 | 1 | 16 | 1.0 |
| 17 | Sample | Sample #6 | 1 | 17 | 1.0 |
| 18 | Sample | Sample #7 | 1 | 18 | 1.0 |
| 19 | Sample | Sample #8 | 1 | 19 | 1.0 |
| 20 | Sample | Sample #9 | 1 | 20 | 1.0 |
| 21 | Sample | Sample #10 | 1 | 21 | 1.0 |
| 22 | QC | Sample #1 Duplicate | 1 | 12 | 1.0 |
| 23 | QC | Laboratory Control Spike | 1 | 22 | 1.0 |
| 24 | QC | Check Standard | 1 | 9 | 1.0 |

Minimum of 3 calibration standards to be run with daily analyses

## APPENDIX OLS MLD073 A3
## Target Compounds Validated by MLD073

| Compound | Standard µg/mL | LOQ µg/mL |
|---|---|---|
| Levoglucosan (1,6-anhydro-β-D-glucopyranose) | 0.5 - 10 | 0.1 |
| Mannsoan (1,6-anhydro-β-D-mannopyranose) | 0.5 - 10 | 0.1 |
| Galactosan (1,6-anyhdro-β-D-galactopyranose) | 0.5 - 10 | 0.1 |

## APPENDIX OLS MLD073 A4
## Replicate LOD Determination

| | | | Galactosan | Mannosan | Levoglucosan |
|---|---|---|---|---|---|
| | Replicate | Conc. | ug/mL | ug/mL | ug/mL |
| LOD #1 | 1 | 0.050 | 0.074 | 0.067 | 0.064 |
| LOD #2 | 2 | 0.050 | 0.076 | 0.068 | 0.063 |
| LOD #3 | 3 | 0.050 | 0.078 | 0.071 | 0.068 |
| LOD #4 | 4 | 0.050 | 0.082 | 0.066 | 0.071 |
| LOD #5 | 5 | 0.050 | 0.077 | 0.062 | 0.068 |
| LOD #6 | 6 | 0.050 | 0.061 | 0.054 | 0.057 |
| LOD #7 | 7 | 0.050 | 0.070 | 0.054 | 0.065 |
| Average | | | 0.074 | 0.063 | 0.065 |
| Median | | | 0.076 | 0.066 | 0.065 |
| Std.Dev. (σ) | | | 0.0068 | 0.0068 | 0.0045 |
| $t_{01,2}$ = | 3.143 | | | | |
| LOD | $t_{01,2} * \sigma$ | | **0.0214** | **0.0213** | **0.0142** |
| LOQ | 10*σ | | **0.068** | **0.068** | **0.045** |
| Method RL | | | **0.100** | **0.100** | **0.100** |
| | | | | | |
| Criteria (LOQ<RL) | | | yes | yes | yes |
| <5xRL | | | OK | OK | OK |

## APPENDIX OLS MLD073 A5
## Initial Control Values and Control Limits

| Compound | Galactosan | Levoglucosan | Mannosan |
|---|---|---|---|
| Expected Conc. (µg/mL) | 5 | 5 | 5 |
| Number of Control Samples | 20 | 20 | 20 |
| | | | |
| Median | 4.522 | 4.198 | 4.827 |
| sd | 0.494 | 0.818 | 0.636 |
| %RSD | 10.92% | 18.97% | 18.97% |
| Adj.sd | 0.494 | 0.818 | 0.636 |
| | | | |
| UCL | 6.006 | 6.762 | 6.781 |
| UWL | 5.512 | 5.945 | 6.145 |
| LWL | 3.535 | 2.674 | 3.599 |
| LCL | 3.041 | 1.857 | 2.962 |

## APPENDIX OLS MLD073 A6
## Recommended Corrective Actions for QC Failures

| QC Samples | Corrective Action |
|---|---|
| Blanks must be less than LOQ. | If greater than LOQ, reanalyze all samples in batch. Check instrument and method materials for possible contamination. |
| Controls must be within the established limits. | If controls are outside the limits, all samples in the batch must be reanalyzed. If not possible to reanalyze, that compound must be invalidated for each sample in the batch. |
| All duplicate results must be within 30% of original results | If duplicates have >30% difference, all samples in the batch must be reanalyzed. If not possible to reanalyze, those compounds that were outside the limits must be invalidated. |
| Laboratory Control spike results must be within 30% of the expected value. | If spike results are outside the limits, all samples in the batch must be reanalyzed. If not possible to reanalyze, that compound must be invalidated for each sample in the batch. |

## APPENDIX OLS MLD073 A7
## Revision History

| Revision Number | Revision Date | Revision Made |
|---|---|---|
| 0 | 5/18/15 | New SOP |
| | | |
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## APPENDIX OLS MLD073 A8
## Review History

| Reviewed By | Date Reviewed | Changes Needed | Addendum Added or Revision Made? (Y/N/NA) |
|---|---|---|---|
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