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
Revision 1.0

Version 1.0 Approval Date: December 1, 2014

Prepared By:

Verna Brock
Air Pollution Specialist — Date: 4-4-15

Approved By:

Kathleen Gill
Manager, Organics Laboratory Section — Date: 8/4/2015

Michael Werst
Chief, Northern Laboratory Branch — Date: 8/4/15

Mike Miguel
Chief, Quality Management Branch — Date: 8/4/15

DISCLAIMER: Mention of any trade name or commercial product in this Standard Operating Procedure (SOP) does not constitute endorsement or recommendation of this product by the Air Resources Board (ARB). Specific brand names and instrument descriptions listed in the SOP are equipment used by the ARB laboratory. Any functionally equivalent instrumentation can be used. This method is restricted to use by or under direct supervision of analysts experienced in the use of air sampling methods and analysis by gas chromatography/mass spectrometry (GCMS).

TABLE OF CONTENTS

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-5ms, 30m x 0.25mm (0.25µm) or equivalent

3.3 MS Detector: capable of scanning the mass range from 100 m/z 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 250µL 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, 1.5mL, 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): 10µL, 25µL, 250µL 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 Pyridine, CAS No. 110-86-1, such as Aldrich catalog # 27,040-7.

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

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

4.8 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 STANDARD PREPARATION

6.1 Calibration and Spike Standards

6.1.1 Individual stock standard solutions are prepared by dissolving each reagent grade solid compound 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 100 µg/mL 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.

6.1.3 Working standards: The following table lists the dilutions used to prepare the working standards. Working standards are made by combining each required amount of composite standard diluted with carbonyl-free acetonitrile. The working standards are prepared in GC autosampler vials. These standards are prepared prior to sample analysis and are disposed of after one month or when degradation is observed.

Working Standard Conc. (µg/mL)	Final Dilution Volume (mL)	Composite Standard Conc. (µg/mL)	Volume Needed (µL)
0.5	1.0	100	5
1	1.0	100	10
2	1.0	100	20
4	1.0	100	40
6	1.0	100	60
8	1.0	100	80
10	1.0	100	100

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 second source supplied reagent grade solid compounds. The control stock standards are used to prepare a composite control standard, as described in section 6.1.2. A mixed working control standard is prepared from the composite control standard at a concentration of approximately 5.00 µg/mL of each compound.

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 composite standard solutions, including calibration and control, are stable for 11 months when stored properly.

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, an unexposed $PM_{2.5}$ filter is extracted and used as a filter blank.

For each batch of ten samples, a matrix spike sample is prepared by pipetting 40 µL of the 100µg/mL composite standard (Section 6.1.4) onto an unexposed $PM_{2.5}$ filter before extracting it using the same procedure 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.

Prior to analysis, a 100µL aliquot of each extracted sample, spike, or blank is placed in a 250µL vial insert contained in an autosampler vial. The Teflon lined cap is secured on the vial. 20µL of pyridine is added to the 100µL aliquot through the septa in the vial cap, followed by 20µL of silanizing reagent. 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 be completed within 24 hours of derivatization.

Standards are made by taking 100µL 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 or 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, are 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.

8.2 GC/MS Initial Setup

Typical gas chromatograph, mass spectrometer settings and programs may be found in Appendix MLD073 A1. Specific steps for instrument and sample preparation along with current instrument parameters and programs can be found in work instruction OLS-WI-073.

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
System blank
Filter blank
Sample set (no more than 10)
Bracketing Standards (as needed)
Sample Replicate (usually first sample in set is reanalyzed)
Matrix Spike
Check Standard
Solvent blank
Any other lab samples such as dilutions
Check Standard

A typical analytical run is shown in Appendix MLD073 A2. Because of the short analytical run time and use of an auto-injector it is suggested that most injections be done in duplicate in case of injection problems. The average of the two injections would be used as data for record.

9.0 QUALITY CONTROL

9.1 Blanks

9.1.1 A system blank must be analyzed before any sample is run. The system blank is carbonyl-free acetonitrile which has been taken through the derivatization process with the sample set without a $PM_{2.5}$ Teflon filter. 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 system blank is included with ten air samples.

9.1.2 A filter 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. One filter blank is extracted and analyzed for every ten samples.

9.1.3 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.)

9.1.4 A field blank is an unexposed $PM_{2.5}$ Teflon filter shipped to the laboratory by sampling personnel. They are taken through the extraction/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 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 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 0.5µg/mL to 10µg/mL. A multipoint analysis must be performed with each sample set prior to analysis of samples. The multipoint analysis is injected in duplicate, and the average of the duplicate injections is used to determine linearity. 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 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 20 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. 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 the lowest calibration standard.

The lowest standard should typically be at or below 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 points. The average and standard deviation for the 20 control data points for each analyte are then determined. If percent relative standard deviations (RSDs) 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. To calculate the limits the percent RSD (or five percent) is multiplied by three and the value obtained by multiplying the average concentration by this factor is added and subtracted from the average to establish the upper and lower control limits, respectively. A similar calculation using a factor of two is used to determine the upper and lower warning limits. Initial control limits are shown in Appendix MLD073 A5.

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 sample 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 control sample is changed from the one used to establish the control limits, new control limits must be established.

9.7 Duplicates

Duplicate samples are analyzed with each sample set of ten. Precision is measured by the percent difference (% D) of the sample or standard duplicate analyses. Maximum allowable % D for the duplicate sample analyses are +/- 25 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 +/- 25 percent, the analysis is discontinued and the cause of the problem is investigated. All associated samples must be reanalyzed after correcting the problem.

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 matrix spike sample 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 matrix spike sample is prepared and analyzed with every ten samples. Matrix spike samples can suffer from matrix interferences 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% 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 An analytical set must be analyzed within 24 hours following derivatization. Derivatized compounds' stability may become questionable 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 as required prior to each set of analytical runs to remove these contaminants. The bake out temperature should 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 in µg/mL. Sample concentrations that exceed the daily calibration concentration level by more than 10 percent must be diluted and reanalyzed.

Ambient air concentrations are reported as µg/m^3 and are calculated as:

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

It is recommended that duplicate injections are performed for every blank, standard, and sample in a sample set. If the analysis is performed in this manner, the average of the duplicate injections will be used as the analyzed value in calculating concentrations unless the percent RSD is greater than 25 percent. If the percent RSD exceeds 25 percent for samples, blanks, or controls, the cause will be investigated. If the cause is determined to be attributed to an injection error or another obvious random issue, the single acceptable analysis will be used as the analyzed value; otherwise, the sample will be reanalyzed. 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 a reference fragments, generally supplied by one of the analytical set's standards and their retention time to this standard's referenced retention time. Both of these references are stored in the method.

12.3 The integrated calibration standard areas for the primary quantitation ions are used to calibrate the method for both retention time and concentration during data processing. The latter is based on the peak areas and the known analyte concentration in the standards. Using this instrument standardization routine the samples, blanks, controls, and spikes' concentrations are calculated.

12.4 All QC and sample results are verified by the bench chemist and then sent to the Laboratory Information Management System (LIMS) for archive and reporting. 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.

14.0 REFERENCES

14.1 MLD QC Manual

14.2 OLS-MLD073-WI-01

15.0 APPENDIX

Appendix OLS MLD073 A1	GC/MS Instrument Method for MLD073
Appendix OLS MLD073 A2	Typical Analytical Sequence for MLD073
Appendix OLS MLD073 A3	Target Compounds Validated by MLD073
Appendix OLS MLD073 A4	Replicate LOD Determination
Appendix OLS MLD073 A5	Initial Control Values and Control Limits
Appendix OLS MLD073 A6	Recommended Corrective Actions for QC Failures
Appendix OLS MLD073 A7	Revision History
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	2	2	1.0
3	Calibration	Standard 2	2	3	1.0
4	Calibration	Standard 3	2	4	1.0
5	Calibration	Standard 4	2	5	1.0
6	Calibration	Standard 5	2	6	1.0
7	Calibration	Standard 6	2	7	1.0
8	Calibration	Standard 7	2	8	1.0
9	Sample	Control A	2	9	1.0
10	Blank	System Blank	2	10	1.0
11	Blank	Filter Blank	2	11	1.0
12	Sample	Sample #1	2	12	1.0
13	Sample	Sample #2	2	13	1.0
14	Sample	Sample #3	2	14	1.0
15	Sample	Sample #4	2	15	1.0
16	Sample	Sample #5	2	16	1.0
17	Sample	Sample #6	2	17	1.0
18	Sample	Sample #7	2	18	1.0
19	Sample	Sample #8	2	19	1.0
20	Sample	Sample #9	2	20	1.0
21	Sample	Sample #10	2	21	1.0
22	Sample	Sample #1 Duplicate	2	12	1.0
23	Sample	Matrix Spike	2	22	1.0
24	Sample	Check Standard/Control B	2	9	1.0

Duplicate injection for standards and samples is recommended
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

Compound	Replicate	Galactosan µg/mL	Mannosan µg/mL	Levoglucosan µg/mL
Run	1	0.050	0.023	0.015
Run	2	0.045	0.020	0.013
Run	3	0.042	0.018	0.010
Run	4	0.044	0.021	0.014
Run	5	0.044	0.016	0.011
Run	6	0.042	0.016	0.008
Run	7	0.043	0.016	0.009
Run	8	0.040	0.016	0.009
Average		0.044	0.018	0.011
Median		0.044	0.017	0.011
Std.Dev. (σ)		0.003	0.003	0.003
$t_{01,1}$ =	2.998			
LOD	$t_{01,1}$ * σ	**0.0089**	**0.0083**	**0.0078**
LOQ	10*LOD	**0.089**	**0.083**	**0.078**
S/N		837	59	84
Std.Dev. (σ)		160	22	18
%RSD		19.1%	37.3%	21.4%
IDL (SN/10)		0.001	0.003	0.001

$t_{01,1}$ = single tail 99% confidence interval

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	4.757	5.087	4.056
sd	0.165	0.231	0.225
%RSD	0.034	0.046	0.055
Adj.sd	0.240	0.253	0.225
UCL	5.525	5.826	4.770
UWL	5.285	5.572	4.545
LWL	4.324	4.559	3.645
LCL	4.084	4.306	3.420

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 shall be reanalyzed. If not possible to reanalyze, that compound shall be invalidated for each sample in the batch
All duplicate results must be within 25% of original results	If duplicates have >25% difference, all samples in the batch shall be reanalyzed. If not possible to reanalyze, those compounds that were outside the limits shall be invalidated

APPENDIX OLS MLD073 A7
Revision History

Revision Number	Revision Date	Revision Made
1.0	12/1/14	New SOP

APPENDIX OLS MLD073 A8
Review History

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

Air Resources Board

Mary D. Nichols, Chair
1001 I Street • P.O. Box 2815
Sacramento, California 95812 • www.arb.ca.gov

Matthew Rodriquez
Secretary for
Environmental Protection

Edmund G. Brown Jr.
Governor

TO: Kathy Gill, Manager
Organic Laboratory Section

FROM: Patrick Rainey, Manager
Quality Management Section

DATE: December 7, 2016

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

Thank you for your submission of the addendum (see attached) to the Standard Operating Procedure (SOP) for the Analysis of Levoglucosan, Mannosan, and Galactosan in Ambient Air Using Gas Chromatography/Mass Spectrometry (MDL 073 R1). The Air Resources Board's (ARB) Quality Management Branch reviewed the SOP Addendum and determined that it covers all of the required elements. The addendum is approved.

Please direct comments or questions to Kyle Vagadori at 916-445-9391 or by email at kyle.vagadori@arb.ca.gov.

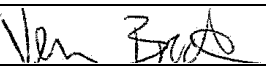
Attachment

cc: Mike Miguel, Chief
Quality Management Branch

Kyle Vagadori
Quality Management Section

QUALITY MANAGEMENT DOCUMENT ADDENDUM

Section 1. ARB Document
☐ Quality Management Plan (QMP)
☐ Quality Assurance Project Plan (QAPP)
☒ Standard Operating Procedure (SOP)

Section 2. Information		
Submitter Name:	Verna Brock	
Submitter Signature/Date:		12-7-16

Section 3. Document Title (*specify exact title, revision #, and date of ARB Document(s) that your District proposes to modify)*	**Date**
SOP MLD073 Revision Number 1.0	December 1, 2014

Section 4. Revision(s)
(specify exact section(s), page number(s) and language in existing ARB document that will be modified and then specify modification (including any spreadsheets or forms).

Section 4.0, page 4

Current 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

Section 4. Revision(s)
(specify exact section(s), page number(s) and language in existing ARB document that will be modified and then specify modification (including any spreadsheets or forms).

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

ARB modification to section 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

4.8 Methanol, reagent grade, used for post-injection syringe wash

4.9 Nanopure water, used in conjunction with methanol for post-injection syringe wash

Section 7.0, page 7

Current 7.0, 3rd paragraph

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

4th paragraph

Section 4. Revision(s)
(specify exact section(s), page number(s) and language in existing ARB document that will be modified and then specify modification (including any spreadsheets or forms).

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 µL 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.

ARB modification to section 7.0

3rd paragraph

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

4th paragraph

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. 10 µL of silanizing reagent is added via syringe to the 100 µL 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.

Section 9.3, page 9

Current 9.3, first paragraph

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.

Section 4. Revision(s)
(specify exact section(s), page number(s) and language in existing ARB document that will be modified and then specify modification (including any spreadsheets or forms).

ARB modification to 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 0.1 µg/mL to 10 µg/mL. A multipoint analysis must be performed with each sample set prior to analysis of samples. A full range multipoint calibration (0.1µg/mL - 10µg/mL) must be performed quarterly, at a minimum. 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.

Appendix OLS MLD073 A1

Current 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

Section 4. Revision(s)
(specify exact section(s), page number(s) and language in existing ARB document that will be modified and then specify modification (including any spreadsheets or forms).

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
Ionization

Precursor Ion (m/z)	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

Section 4. Revision(s)
(specify exact section(s), page number(s) and language in existing ARB document that will be modified and then specify modification (including any spreadsheets or forms).

Multiplier Offset: 0 volts
Count Threshold: 1

ARB modification to Appendix I

Thermo AI/AS 1310 Autosampler

Sampling
Syringe Size: 10 uL
Sampling: Sample volume (µL): 1.00
Plunger strokes: 3
Viscous sample: yes
Sampling depth in vial: Bottom

Injection
Injection depth: Standard
Pre-Inj dwell time (s): 0.0
Post-Inj dwell time (s): 0.0

Washes
Pre-Inj solvent: Acetonitrile (vial A)
Pre-Inj solvent cycles: 3
Pre-Inj solvent: Acetonitrile (vial B)
Pre-Inj solvent cycles: 3
Sample rinses: 0
Post-Inj solvent: Methanol (vial C)
Post-Inj solvent cycles: 3
Post-Inj solvent: 50:50 Methanol:H_2O (vial D)
Post-Inj solvent cycles: 3

Thermo TRACE 1300 Series GC Method

Oven Method:
Initial temperature: 100 C
Initial hold time: 2.00 min
Number of ramps: 2
Ramp 01 rate: 10.0 C/min
Ramp 01 final temperature: 200.0 C
Ramp 02 rate: 45.0 C/min
Ramp 02 final temperature: 280.0 C
Ramp 02 hold time: 5.00 min

Section 4. Revision(s)
(specify exact section(s), page number(s) and language in existing ARB document that will be modified and then specify modification (including any spreadsheets or forms).

S/SL – Front Method
S/SL mode: Splitless
Temperature: 275 C
Split flow enable: On
Split flow: 50.0 mL/min
Splitless time: 1.00 min
Purge flow: 5.0 mL/min
Constant septum purge: On
Carrier mode: Constant Flow
Carrier flow: 1.4mL/min
Vacuum compensation: On
Backflush start time: 1.50 min

Thermo ISQ Series Method
Method type: Acquisition – General
MS transfer line temperature: 280 C
Ion source temperature: 285 C
Ionization mode: EI
Run completion: stop after 9.5 min
Segment #1 start time: 7.5 min
SIM Scan #1 Mass: 204 amu
  Width: 1 amu
  Dwell time: 0.2 sec
SIM Scan #2 Mass: 217 amu
  Width: 1 amu
  Dwell time: 0.2 sec
SIM Scan #3 Mass: 333 amu
  Width: 1 amu
  Dwell time: 0.2 sec

Appendix OLS MLD073 A3

Current Appendix OLS MLD073 A3

APPENDIX OLS MLD073 A3
Target Compounds Validated by MLD073

Section 4. Revision(s)
(specify exact section(s), page number(s) and language in existing ARB document that will be modified and then specify modification (including any spreadsheets or forms).

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

ARB Modification to Appendix OLS MLD073 A3

APPENDIX OLS MLD073 A3
Target Compounds Validated by MLD073

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

Section 5. Justification for Deviation(s) *(provide explanation of why modification(s) to existing ARB document is necessary)*
Modifications reflect results of method development studies aimed at reducing carryover, streamlining standard preparation and calibration, and extending hold times.

Section 6. Attachment(s) ☐ *(specify attachment titles and number of pages, include modified spreadsheets or forms)*	**# of Pages**

Section 7. ARB Approval		
Name/Phone Number:	Kathy Gill	916 445-9483
Title:	Manager, Organics Laboratory Section	
Signature/Date:	Kathy Gill	12/7/16
Addendum Number	A16 MLD 073	

Completed form must be scanned/emailed or mailed to:

Ms. Kathy Gill
1927 13th Street, P.O. Box 2815
Sacramento, California 95811
kgill@arb.ca.gov