Title: Determination of Levoglucosan and other Carbohydrates in Atmospheric Aerosols by Ion Chromatography with Pulsed Amperometric Detection (IC-PAD)		Copy No: ##
Method No.: 6.12/2.0/M	Effective Date: February 04, 2013	Location: ###

QSM Approval: _____

Determination of Levoglucosan and other Carbohydrates in Atmospheric Aerosols by Ion Chromatography with Pulsed Amperometric Detection (IC-PAD)

1. INTRODUCTION AND SCOPE

1.1. This method is applicable to the determination of levoglucosan and other carbohydrates (listed in Table 1) commonly found in atmospheric aerosols.

1.2. Method detection limits (MDLs) for levoglucosan and other analytes of interest are in the range of low ng/filter (based on 15mL extraction volume). Expanded measurements uncertainties (k = 2) at the concentrations above the method quantitation limits (MQLs = 3 x MDLs) are in the range of 10-15%.

1.3. This method is recommended for use only by, or under the supervision of, analysts experienced in the use of ion chromatography and in the interpretation of the resulting ion chromatograms.

TABLE 1. Analytes determined by this method

Anhydrosugars	Levoglucosan (1,6-anhydro-β-d-glucose, 1,6-anhydro-β-d-glucopyranose)
	Mannosan (1,6-anhydro-β-d-mannopyranose)
	Galactosan (1,6-anhydro-β-d-galactopyranose)
Sugar alcohols	Arabitol
	Mannitol
Monosaccharides*	Mannose
	Galactose
	Glucose
	Fructose

*For screening only

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2. SUMMARY OF METHOD

2.1. IC-PAD combines pulsed amperometric detection with the separation capabilities of ion exchange resins. Levoglucosan and other analytes of interest are separated and measured using a system comprising guard column, separation column, and electrochemical detector. The concentrations of all analytes are determined by measuring electrical current generated by their oxidation on gold electrode. The analytes are identified by their retention times as compared to the retention times of known standards.

2.2. The **Carbohydrates_MA1** program is used for automatic determination of all analytes.

Experimental Conditions:

Separation column: CarboPac-MA1 (4 mm x 250 mm)

Guard column: CarboPac-MA1 (4 mm x 50 mm)

Eluent: 500 mM NaOH (isocratic)

Eluent flow-rate: 0.4 mL/min

Sample loop: 50 µL

Detector: ED with Amperometry Cell

Working Electrode - Gold

Reference Electrode – pH-Ag/AgCl

TABLE 2. Waveform program

Time (sec)	Potential (V)	Integration
0.00	+ 0.15	
0.20	+ 0.15	Begin
0.40	+ 0.15	End
0.41	- 2.00	
0.42	- 2.00	
0.43	0.60	
0.44	- 0.15	
0.50	- 0.15	

3. INTERFERENCES

3.1. Interference can be caused by substances with retention times that are similar to, and overlap with, those of the analytes of interest (e.g. amines and organic sulfur species).

4. SAMPLE REQUIREMENT

4.1. Consult SOP 6.06/*.*/S for the storage conditions and extraction procedure.

5. EQUIPMENT and SUPPLIES

5.1. All ion chromatography equipment and software are from one manufacturer (Dionex, Sunnyvale, CA, USA). The Dionex IC system consists of:

5.1.1. Gradient pump, electrochemical detector, guard and separation columns.

5.1.2. Automated sample changer (AS or equivalent) carrying sample vials with sample size 2 mL.

5.1.3. Personal computer containing the operating and processing software (Chromeleon 6.**, Dionex, Sunnyvale, CA, USA).

5.2. Millipore RiO-30 (with UV lamp for bacteria elimination) and Super-Q water purifying systems.

5.3. Balances:

5.3.1. Analytical balance (Mettler AT 400 or equivalent) - sensitivity 0.1 mg;

5.3.2. Technical balance (Mettler PR 1200 or equivalent) – sensitivity 10 mg.

5.4. Ultrasonic bath or mechanical shaker.

5.5. Micropipettes and Bottle Dispensers.

5.6. Glassware, Sample Vials and Caps: Note: All glassware used in the laboratory is Class A (consult SOP 6.02/*.*/S for cleaning of all labware used in the laboratory).

6. REAGENTS and STANDARDS

Unless otherwise indicated, it is intended that all reagents are analytical grade, where such specifications are available; otherwise, the best available grade is used. All solutions are prepared in deionized water.

6.1. **Reagent water:** To prevent gas bubbles from forming in the reagent line and pump, use high-purity deionized, degassed water (DI water, resistance >18 MOhm cm) for

the preparation of solutions and for rinsing glassware. Deionized water is obtained from Millipore RiO-30 and Super-Q water purifying systems (or equivalent).

6.1.1. To degas water, fill 4L reservoir with fresh deionized water and purge with helium for approximately 20 minutes.

6.2. **0.5 M Sodium Hydroxide (NaOH) eluent solution**

6.2.1. Carefully pipette 26.1 mL of 50% (w/w) NaOH to a 1-liter plastic volumetric flask containing about 800 mL of degassed DI water. Make sure to allow the 50% (w/w) NaOH on the outside of the pipette tip to drip off before transferring the contents to the volumetric flask.

6.2.2. Dilute to the mark with degassed DI water. Add a stirring bar and stir gently for 2 minutes.

6.2.3. Carefully transfer the solution into the polypropylene Dionex eluent bottle and keep blanketed under helium or nitrogen at 5-8 psi at all the time.

Note:

Avoid the introduction of carbon dioxide from air into the 50% (w/w) NaOH or the degassed, DI water being used to make eluent.

- Do not shake the 50% (w/w) NaOH stock.
- Pipette the required aliquot from the middle of the stock solution where sodium carbonate is least likely to have formed.
- Do not pipette from the bottom where sodium carbonate precipitate may have fallen.
- Close the bottle immediately after each use and leave open for the shortest time possible.
- Use fresh bottle and discard if sodium carbonate precipitation is evident. (Usually about 2/3 of the bottle can be used before a fresh bottle is needed.)

6.3. Standards

6.3.1. **Mixed Standard Solutions S-1, S-2 and S-3.** Prepare mixed standard solutions from single carbohydrates standard solutions (1000 mg/L each) in 50 mL volumetric flask with degassed DI water. Mixed Standard Solutions should be prepared within time limits specified in Table 3, or when any deterioration is observed, and stored in the refrigerator at 4±2°C. Consult SOP 6.03/*.*/S regarding preparation, storage and use of the standard solutions in the laboratory.

TABLE 3. Preparation of Mixed Standard Solutions

	Stock S-1 *(fresh every 12 months)*		Stock S-2 *(fresh every 6 months)*		Stock S-3 *(fresh every 6 months)*	
Analyte:	µL of single stock (in 50 mL)	*Concentration [mg/L]*	µL of single stock (in 50 mL)	*Concentration [mg/L]*	µL of single stock (in 50 mL)	*Concentration [mg/L]*
Levoglucosan	1000	*20*	-	-	-	-
Arabitol	-	-	500	*10*	-	-
Mannosan	1000	*20*	-	-	-	-
Mannitol	-	-	500	*10*	-	-
Mannose	-	-	-	-	500	*10*
Galactosan	1000	*20*	-	-	-	-
Glucose	-	-	-	-	500	*10*
Galactose	-	-	-	-	500	*10*
Fructose	-	-	-	-	2000	*40*

6.3.1.1. Calibration Standards

Calibration Standards should be prepared within time limits specified in Table 4, or when any deterioration is observed. Store Cal-5 and Cal-6 in the refrigerator at 4±2°C.

Note: The concentration of calibration standards may be adapted to the sample concentration range (See Section 7.1).

TABLE 4. Preparation of Calibration standards and their concentrations.

	Cal-0	Cal-1	Cal-2	Cal-3	Cal-4	Cal-5*	Cal-6**
	(Fresh prior to analysis)					*(Fresh every 6 months)*	
Vol. Flask [mL]	10	50	50	50	50	50	50
Stock S-1 [µL]	0	50	125	250	625		
Stock S-2 [µL]	0	50	125	250	625		
Stock S-3 [µL]	0	50	125	250	625		
Concentration [µg/L]							
Levoglucosan	0	20	50	100	250	500	1000
Arabitol	0	10	25	50	125		
Mannosan	0	20	50	100	250		
Mannitol	0	10	25	50	125		
Mannose	0	10	25	50	125		
Galactosan	0	20	50	100	250		
Glucose	0	10	25	50	125		
Galactose	0	10	25	50	125		
Fructose	0	40	100	200	500		

*Cal-5 – Dilute 25 µl of levoglucosan standard (1000 mg/L) with degassed, deionized water in 50mL volumetric flask

**Cal-6 - Dilute 50 µl of levoglucosan standard (1000 mg/L) with degassed, deionized water in 50mL volumetric flask

6.3.2. Control Standards

Control Standards are prepared from the commercially available reference stocks and/or certified standards (e.g. o2si Ssmart Solutions, Delta Scientific Laboratory Products Ltd.). Store all control standards and reference standards in the refrigerator at 4±2°C.

- **CS-S1:** Levoglucosan, Mannosan, Galactosan – 0.050 mg/L each; Arabitol, Mannitol – 0.025 mg/L each. Prepare CS-S1 by diluting 250 µL of O2Si standard 116564-05 and 125 µL of O2Si standard 116566-05 in 50 mL volumetric flask with degassed, deionized water. CS-S1 should be **prepared fresh daily**.

- **CS-S2:** Levoglucosan, Mannosan, Galactosan, Arabitol, Mannitol, Mannose, Glucose, Galactose, Fructose – 0.100 mg/L each. Use O2Si standard 116565-03. The expiry of CS-S1 is specified by the manufacturer.

- **CS-S3:** Levoglucosan, Mannosan, Galactosan – 0.250 mg/L each. Prepare CS-S2 by diluting 1250 µL of O2Si standard 116564-05 in 50 mL volumetric flask with degassed, deionized water. CS-S2a should be **prepared fresh every 6 months**, or when degradation is observed.

7. CALIBRATION AND STANDARDIZATION

7.1. Calibration of the instrument is performed using working standards before analysis of the first set of samples in any given week. Calibration may also be necessary after unusual performance of the instrument (e.g. electrode failure). The instrument does not need to be re-calibrated during the remainder of the week as long as the calculated concentrations of verification and control standards are within the specified limits (See Section 10.2).

7.1.1. The calibration uses the external standard quantitation method. The linear dynamic range is established using a multi-point calibration curve constructed for every target analyte. If the coefficient of correlation for the calibration curve is better than 0.995, linearity for the working range is established. A high-concentration calibration curve includes Cal-5, Cal-6, and is used for highly concentrated samples.

7.2. Verify calibration accuracy by analyzing quality control standards at different concentration ranges (CS-S1/CS-S2/CS-S3). If the response varies from expected values by more than the warning limits (see Section 10.2), fresh control standards must be prepared. If the results are still more than the specified limits, a new calibration curve must be prepared. If the results still exceed the limits, the analyses should be terminated until the source of the problem is identified and corrected.

7.3. Verify the system calibration daily throughout the analysis by intermittently analyzing control standard (i.e.: every fifteen samples; See Section 10.2). If the concentrations of determined analyte fall outside acceptable limits (See Appendix C), then analyte is deemed to be "out of control". All samples following the last acceptable control standard should be reanalyzed.

7.4. Verify system stability by analyzing a verification standard (VS-S = Cal-3) on each working day. The concentration must fall within warning limits (See Section 10.2). If results fall outside the limits, analyze an additional aliquot of the standard. If unacceptable results persist, then recalibrate the instrument and reanalyze all samples measured after the last acceptable control standard.

8. ANALYSES

8.1. Make sure that all necessary maintenance procedures of instruments are done before batch analysis.

8.1.1. Check gas cylinder to see if it is turned on and if the pressure is > 300 psi.

8.1.2. Check eluent every day to see if there is enough to run.

8.2. Polish the working electrode (see instrument manual for instructions), assemble the detector cell, start up the instrument and equilibrate the system for at least 24h. For full-week analysis, the electrode should be polished on Friday of the preceding week and allowed to equilibrate over weekend. Check background current and backpressure after the system stabilizes (use control chart to record values).

8.3. Prepare the sequence for the automated run (Appendix A), which includes samples, calibration standards, method blank (**MetBlank**), spiked blank filters (**SP-MDL** and **SP-VSS**), control standards (**CS-S**), verification standards (**VS-S**), duplicate samples and **NIST 1649b** extract.

8.3.1. The sample files and sequence are named according to the batch name.

8.4. Extract filters (Consult SOP 6.06/*.*/S).

8.4.1. Prepare spiked blank filters (SP-MDL & SP-VSS).

- **SP-MDL**: Place a blank Teflon filter in a labeled extraction bottle. Wet filter with 100 µL of isopropanol and spike with 15mL of the lowest calibration standard (Cal-1). Sonicate SP-MDL together with samples.
- **SP-VSS**: Place a blank Teflon filter in a labeled extraction bottle. Wet filter with 100 µL of isopropanol and spike with 15mL of the verification standard (Cal-3). Sonicate SP-VSS together with samples.

8.4.2. Prepare **NIST1649b** extract: weigh approximately 10mg of NIST 1649b standard material and record the exact weight in the analyst notebook. Transfer the weighed material to a scintillation vial, wet with 100µL of isopropanol, add 15 mL of degassed DI water and sonicate for 30min. Filter the extract through 0.45µm syringe filter.

8.5. Fill the sample vials with the calibration standards, quality control and unknown samples and place them in the sample tray according to the schedule (see Appendix A).

8.5.1. Start sequence run.

8.6. After the run of Cal-3 (system performance check), compare the values for retention time and peak area (levoglucosan) to those of the most recent run. If the value of retention time or peak area exceeds a ± 5% and 30%, respectively, stop the run and eliminate the source of the problem (e.g. prepare new working standards, and/or eluent).

8.7. Check system calibration and stability daily through the analysis as described in Section 7.

9. DATA PROCESSING

9.1. Before reprocessing, copy all files from the lab computer to the analyst's office computer, and create a backup file (backup files should be stored in: Inorg:\ION ANALYSIS LAB\IC_raw_data_backup). After making sure that all data has been copied and backed up, delete it from the lab computer.

9.2. Prepare a calibration curve for each analyte using Chromeleon Chromatography Software by plotting instrument response (peak area) against standard concentration (including Cal-0). Check all calibration curves (See Section 7).

9.3. Reprocess all quality control samples and check accuracy of the analytes. Results for control and verification standards should be within specified limits (see Section 10.2). If the results are outside limits, check again the calibration curves and correct them if required. Flag the results that are out of limits. If two out of three successive results

are outside warning limits, control standards and affected samples must be reanalyzed.

9.4. Reprocess all unknown samples. Visually check all chromatograms. Any sample exceeding the highest standard should be diluted and reanalyzed.

9.5. Use Excel software to prepare a final report using validated template for the project.

9.5.1. Before reporting to the client, the authorized lab person must check all results (see Appendix C).

9.6. After the final report is completed, approved and password-protected, transfer all files for backup to the Network - Inorg on 'ncr.int.ec.gc.ca\lab\riv' drive - into appropriate directory. Validate the EXCEL templates containing formulas and/or macros at least once a year or after major changes (SOP 2.11/*.*/S). The validated templates should be password-protected (Read Only). Record the file name and the validation date in the designated log book.

10. QUALITY CONTROL

10.1. The QC samples used normally comprise 10-25% of total sample through put.

10.1.1. Typical batch of approximately 90 samples includes various blanks, control/verification standards, and duplicate samples (See Appendix A).

10.1.1.1. Method Blank (MetBlank) – a blank filter in analyte-free water, which undergoes processing identical to that carried out for the samples. The blank results are used to assess contamination.

10.1.1.2. Verification Standard (VS-S) – standard from calibration (Cal-3) is used to monitor system stability.

10.1.1.3. Duplicate – a second aliquot of the same sample is used to evaluate the reproducibility of the laboratory procedure during the run of a single batch.

10.1.1.4. Spike (SP-MDL and SP-VSS) – a blank filter spiked with Cal-1 (SP-MDL) or Cal-3 (SP-VSS) solution, which undergoes processing identical to that carried out for the samples. The spike results are used to calculate recoveries of analytes during the sample processing.

10.1.1.5. NIST1649b extract – 10mg of NIST SRM 1649b which undergoes extraction and processing steps similar to those of the samples.

10.1.1.6. Control standards (CS-S1, CS-S2, CS-S3) – standards prepared from certified standard solutions used to monitor accuracy of the calibration.

10.2. **Data Acceptance**

10.2.1. Control Standards, Verification Standards, and Spikes should be within 10% of target values.

10.2.2. Blanks should be below the method detection limit (MDLs).

10.2.3. Duplicate samples should have the relative percentage difference (RPD) within 10-15% (for samples at concentration higher than the quantitation limit).

10.2.4. If the above data acceptance criteria are not met, specific control standards and samples are repeated.

10.3 All quality control data should be maintained and available for easy reference or inspection.

11. METHOD DETECTION LIMITS and METHOD VALIDATION

11.1. The single laboratory (Particulate Characterization Unit - AAQS) MDLs for the analyzed carbohydrates are calculated based on historical data and/or repeated measurements of SP-MDL (a blank filter spiked with a low concentration standard, not higher than 5-10 times the expected MDL, and carried through all the sample processing steps.

11.1.1 Based on a single day measurements of multiple (at least 7), individually prepared SP-MDL samples, estimate the MDL_{LCS}:

$$MDL_{LCS} = t_{(0.99,\, n)}\, s_{Std}$$

where $t_{(0.99,n)}$ is the Student's t-test for 99% confidence level (single tailed), $n = n_i - 1$ is the degrees of freedom, n_i is the number of repeats, and s_{Std} is their standard deviation (see SOP 2.05/*.*/S).

11.1.2 Based on the historical results of SP-MDL analysis (e.g. latest year), calculate the MDL:

$$MDL = t_{(0.99,\, n-1)}\, s$$

where $t_{(0.99, n-1)}$ is the single tailed Student's test for 99% confidence level and n-1 degrees of freedom, n is the number of repeats, and s_{Std} is their standard deviation.

11.1.3 The single laboratory (Particulate Characterization Unit - AAQS) MDLs for levoglucosan and other analytes of interest are in the range of low ng/filter (based on 15mL extraction volume). For the records of calculated and reported MDLs refer to the latest Method Verification Binder (Room 172).

11.1.4 Method detection limits should be checked at least once a year, and/or after any major modification of the instrument and/or method.

11.2. Method validation/verification is accomplished by using the QC samples described in Section 10 and by intralaboratory comparison (CALA PT option vi). For the records of precision and accuracy of measurements refer to the latest Method Verification Binder (Room 172).

12. ESTIMATION of MEASUREMENT UNCERTAINTY

12.1. The measurement of uncertainty is calculated based on the Type A approach recommended by the CALA (consult SOP 2.10/*.*/S).

12.2. The possible sources of uncertainty and QC data used for uncertainty estimation are listed in Appendix B.

12.3. The overall uncertainties at concentrations above method quantitation limits (MQL = 3xMDL) for determined analytes of interest are in the range of 10-15%. For the records of estimated measurement uncertainties refer to the latest Method Verification Binder (Room 172).

12.4. The uncertainty should be estimated at least once a year, and/or after any major modification of the method. The most recent estimated values should be reported to the client.

The method is fit for the intended use.

13. MAINTENANCE

TABLE 5. Routine maintenance of the IC system.

Pumps	
Pump heads	• Clean annually, rinse before and after every operation • Replace tubing on the peristaltic pump as required • See Product Manual, Dionex Corporation
Pistons	• Rinse before and after every operation (see product manual, Dionex Corporation) • Clean/replace pistons and seals (main and piston wash seal) as required
Check valves	• Prime valves daily (when systems were shut down) • Clean or change when pump has lost prime or it is difficult to re-prime
Seal wash reservoir	• Replace water in the seal wash reservoir every week
Detector/Chromatography Module	
Leaks	• Periodically check for leaks or spills inside modules and for crimping of gas and liquid lines • Eliminate gas leaks whenever drop of the pressure on the gas cylinder regulator exceeds 1000 kPa per week
Amperometry cell/Gold electrode	• Never apply potential to the electrode unless a stream of eluent or water is flowing through the cell • Keep the polished surface of the cell body and the gold spring-loaded contact clean and dry • Polish the working and/or counter electrode when discoloured or degradation in performance noticed (baseline noise, tailing peaks, etc.) • If the system is being shut down for more than 12 h, remove the cell from the detector, disassemble it, and rinse both working and counter electrode with DI water. Leave the cell disassembled on the clean surface, and cover with Kimwipes. • See Product Manual, Dionex Corporation
pH-Ag/AgCl electrode	• Store reference electrode in the solution of saturated KCl when system is being shut down for more than 12 h

	• Regenerate electrode when need it (frit partially dried out) by soaking in a solution of 1M KCl + 1M HCl • See Product Manual, Dionex Corporation
Sample loops	• Clean/replace as required
Injection valves	• Rebuild annually • Clean as required
Columns	• Change bed support, guard and separation columns when necessary (analytical parameters such as retention time, peak shape or backpressure etc. are changing) • See Product Manual, Dionex Corporation
Autosampler	• Daily check syringe for air bubbles • Periodically check alignment of the sampling needle • Annually perform preventive maintenance procedure • See Product Manual, Dionex Corporation

14. APPLICABLE SOPs

2.01/*.*/S "Gravimetric Measurement"

2.05/*.*/S "Method Validation"

2.06/*.*/S "Laboratory Refrigerators and Freezers"

2.10/*.*/S "Estimation of Uncertainty in Chemical Analysis"

2.11/*.*/S "Electronic System Validation and Maintenance"

6.02/*.*/S "Labware Cleaning"

6.03/*.*/S "Preparation, Storage and Use of Standard Solutions"

6.06/*.*/S "Extraction of filters"

19.02/*.*/S "Volumetric Measurement- Micro pipettes and Bottle-Top Dispensers"

19.04/*.*/S "AAQS water purification system"

SWP-001/*.* "Safe Working Procedure and Policies"

15. REFERENCES

15.1. AS Autosampler Operator's Manuals, Dionex Corporation (2008, January)

15.2. ICS-3000 Ion Chromatography System. Operator's Manuals, Dionex Corporation (2006, September)

15.3. Product Manual. CarboPac Combined Products (2005, March)

15.4. Chromeleon. Chromatography Management System. Tutorial and User Manual, Dionex Corporation (2005, April)

15.5 Analysis of Carbohydrates by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD). Technical Note 20, Dionex Corporation (2000, June)

15.6 Optimal Setting for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector. Technical Note 21, Dionex Corporation (1998, October)

15.7 US EPA, Compendium Method IO-3.1, Selection, Preparation and Extraction of Filter Material, June 1999

16. REVISIONS

September 2009: Authors: Michal Suski, Maria Piechowski; New document

December 2009: Reviewers: Michal Suski, Maria Piechowski

Section 4.1 – new; Section 4.2 – removed; Section 6.3.2 – new; Section 8 – vial blank (VialBlank) and spiked blank filter (MetBlank-SP) – added; Section 8.4 – new; Section 9.6 – information about Excel templates validation – added; Section 10.1 – vial blank and spike – added; Section 11.1 – new; Sections 12.3 and 12.4 – new; Section 14 – 2.11 – added; Section 15.7 – added; Appendix A – new; Appendix B, C – edited; Appendix D – removed; Appendix E – changed to D; Appendix F – changed to E.

April - July 2011: Reviewer: Michal Suski

References to AS40 autosampler changed to AS autosampler throughout the text; Section 1.2 – changed to show the range of MDLs, reference to Appendix A removed. Section 5.3.1 & 5.3.2 – 'or equivalent' added to balance descriptions; Section 6 – 'water' replaced with 'deionized water'; Section 6.3.1 – text and chart changed from 2 to 3 mixed standard solutions, chart formatting changed; Section 6.3.2 – Control standards have changed, section

changed to reflect current practice; Section 8.2 – new; Section 8.3 – MBlank-SP1 and MBlank-SP3 replacing MetBlank-SP, NIST 1649B added, ReagBlank and VialBlank removed; Section 8.4.1.1 - MBlank-SP1 added; Section 8.4.2 – new; Section 8.5.1 - removed; Section 8.5.2 - re-numbered to 8.5.1; Section 8.6 – changed to reflect current practices, retention time and peak area acceptance criteria changed; Section 10.1.1.1 & 10.1.1.2 – removed, following sections re-numbered accordingly (10.1.1.1 to 10.1.1.4); Section 10.1.1.5 - new; Section 10.1.1.6 - new; Section 11.1 – "tabulated in Appendix A" removed; Section 11.1.1 – removed, following sections re-numbered accordingly; Section 11.2 – new, 11.2 and 11.3 renumbered 11.2.1 and 11.2.2 respectively; 11.2.3 – new; Section 11.3 – removed; Section 12.3 - 'ions' changed to 'analytes', 10% replaced with 15% to reflect the latest results, section renumbered 12.4; Section 12.4 – renumbered 12.3; Section 13 – changed to reflect the current practices; Appendix A - removed; Appendix B – renamed "A", NIST1649b and MBlank-SP1 added to the schedule, VialBlank and ReagBlank removed, QC's rearranged in the schedule. All following Appendixes renamed accordingly and their references changed throughout the text.

February 2013: Reviewer - Michal Suski

Replaced "store in refrigerator" with "store in refrigerator at 4±2°C" throughout the text; minor formatting changes throughout the document; Section 6.2.1 – preparation of eluent re-written for clarity and consistence with current practices; plastic volumetric flask will be used for preparation of NaOH solutions; Section 6.2.2 – removed; Section 6.3 – calibration standards renamed from 'STD' to 'Cal'; tables for preparation of control standards replaced with text, calibration standards and control standard concentrations adjusted to better suit the concentration of analytes observed in samples; names of spikes changed for consistency with other IC methods; Section 9.5.1 – removed; Section 9.6 – statement "store hard copy of the report for at least one year" removed; Section 11 – expended the description of method used for estimation of MDL's; appendices updated with most recent versions.

Lead Reviewer: Michal Suski Date:
Title: Technologist, Particulate Characterization Unit

Approved by: Ewa Dabek Date:
Title: Head, Particulate Characterization Unit

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APPENDIX A

TABLE A1. Proposed sequence

Name	Type	Program	Method
test	Unknown	Carbohydrates_MA1	Carbohydrates
test	Unknown	Carbohydrates_MA1	Carbohydrates
Cal-0	Standard	Carbohydrates_MA1	Carbohydrates
Cal-1	Standard	Carbohydrates_MA1	Carbohydrates
Cal-2	Standard	Carbohydrates_MA1	Carbohydrates
Cal-3	Standard	Carbohydrates_MA1	Carbohydrates
Cal-4	Standard	Carbohydrates_MA1	Carbohydrates
Cal-5	Standard	Carbohydrates_MA1	Carbohydrates
Cal-6	Standard	Carbohydrates_MA1	Carbohydrates
CS-S1	Validate	Carbohydrates_MA1	Carbohydrates
CS-S2	Validate	Carbohydrates_MA1	Carbohydrates
CS-S3	Validate	Carbohydrates_MA1	Carbohydrates
MetBlank	Unknown	Carbohydrates_MA1	Carbohydrates
Sample 1-16	Unknown	Carbohydrates_MA1	Carbohydrates
Duplicate 1	Unknown	Carbohydrates_MA1	Carbohydrates
CS-S1	Validate	Carbohydrates_MA1	Carbohydrates
Sample 17-32	Unknown	Carbohydrates_MA1	Carbohydrates
Duplicate 2	Unknown	Carbohydrates_MA1	Carbohydrates
SP-MDL	Validate	Carbohydrates_MA1	Carbohydrates
CS-S2	Validate	Carbohydrates_MA1	Carbohydrates
Sample 33-48	Unknown	Carbohydrates_MA1	Carbohydrates
Duplicate 3	Unknown	Carbohydrates_MA1	Carbohydrates
NIST1649b	Unknown	Carbohydrates_MA1	Carbohydrates
Sample 49-64	Unknown	Carbohydrates_MA1	Carbohydrates
Duplicate 4	Unknown	Carbohydrates_MA1	Carbohydrates
VS-S	Validate	Carbohydrates_MA1	Carbohydrates
CS-S3	Validate	Carbohydrates_MA1	Carbohydrates
Sample 65-80	Unknown	Carbohydrates_MA1	Carbohydrates
Duplicate 5	Unknown	Carbohydrates_MA1	Carbohydrates
SP-VSS	Validate	Carbohydrates_MA1	Carbohydrates
Sample 81-96	Unknown	Carbohydrates_MA1	Carbohydrates
Duplicate 6	Unknown	Carbohydrates_MA1	Carbohydrates
VS-S	Validate	Carbohydrates_MA1	Carbohydrates

Title: Determination of Levoglucosan and other Carbohydrates in Atmospheric Aerosols by Ion Chromatography with Pulsed Amperometric Detection (IC-PAD)		Copy No: ##
Method No.: 6.12/2.0/M	Effective Date: February 04, 2013	Location: ###

APPENDIX B

FIGURE B1. Major sources of uncertainty

Uncertainty Sources	QC Data Used
Extraction Conditions	MetBlank SP-MDL SP-VSS
Instrument Drift	Duplicates VS-S
Extraction Volume	Weight of water
Analyst: -Standard preparation -Calibration -Data processing	CS-S1 CS-S2 CS-S3

APPENDIX C

FIGURE C1. Data validation checklist

DATA VALIDATION CHECK LIST

Report Header

Client:
Project:
Number of samples:
Checked/Approved by: **Date:**
Report Prepared:
Report Version:

General Information

Sample Preparation
Analytical Instrument
Analytical Instrument Model #
Method ID
Batch #
Analyst

NOTES:

QA/QC samples checklist

Parameter	QC sample	Sample name	Check (by analyst)
Calibration: - correlation coefficient >99.5%			
QC Standards (recoveries within limits specified in the method)	VS		
	Control Standards		
	Other QCs		
Blanks (less than MDLs)	Vial Blanks		
	Method Blanks		
Spikes (recoveries within limits specified in the method)	MDL spike		
	VS spike		
Trend analysis			
Duplicates (%RPD within limits specified in the method)			

Samples analysis results checklist

Parameter	Check (by analyst)
Unusual results*	

Reporting checklist

Latest Template used for Internal Report	
Latest Template used for the Final Report	
Sample information taken from: (i.e NAPS site ID, Sampling date, PM mass, Actual air volume, lab comments and codes)	
MDLs (latest values)	
Units (both reports)	
Uncertainties (latest estimation)	
Calculations (random samples check)	
Cross-check (final vs internal report)	
Checklist completed and printed	

APPENDIX D

FIGURE D1. Example of a chromatogram

Sample Analysis Report

Sample Name:	Calibration 3	**Sample No.:**	6
Sequence Name:	TB099	**Sample Type:**	standard
Program Method:	Carbohydrates_MA1	**Injection vol.:**	50 µL
Quantitation Method:	Carbohydrates	**Dilution Factor:**	1
Date Time Collected:	20/08/2012 1:02 PM	**Comments:**	
Analyst:	M.Suski	**Method:**	6.12/1.2/M

Peak ED_1 No.	Component ED_1 Name	Retention ED_1 Time	Area ED_1 nC*min	Height ED_1 nC	Amount ED_1 ppb	Modified? ED_1	Amnt.Dev.(rel) ED_1 %
1	Levoglucosan	15.13	0.442	1.005	96.93	*	-3.07
2	Arabitol	16.32	2.289	4.474	198.59	*	-0.70
3	Mannosan	17.50	1.241	2.239	198.20	*	-0.90
4	Mannitol	21.06	0.959	1.463	97.22	*	-2.78
5	Mannose	22.58	0.655	0.933	96.50	*	-3.50
6	Galactosan	23.67	0.933	1.339	196.01	*	-1.99
7	Glucose	25.18	0.798	1.100	95.27	*	-4.73
8	Galactose	27.99	0.871	1.019	94.69	*	-5.31
9	Fructose	29.49	0.745	0.861	382.43	*	-4.39