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High-performance anion-exchange chromatography–mass spectrometry method for determination of levoglucosan, mannosan, and galactosan in atmospheric fine particulate matter

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Abstract Biomass burning has a strong influence on the atmospheric aerosol composition through particulate organic, inorganic, and soot emissions. When biomass burns, cellulose and hemicelluloses degrade, producing monosaccharide anhydrides (MAs) such as levoglucosan, mannosan, and galactosan. Therefore, these compounds have been commonly used as tracers for biomass burning. In this study, a fast water-based method was developed for the routine analysis of MAs, based on high-performance anion-exchange chromatography with electrospray ionization mass spectrometry detection. This method combines simple sample preparation, fast separation, and the advantages of the selective detection with MS. Analysis run was optimized to the maximum separation of levoglucosan, mannosan, and galactosan with 15-min analysis. The validation results indicated that the method showed good applicability for determination of MA isomer concentrations in ambient samples. The limit of detection was 100 pg for levoglucosan and 50 pg for mannosan and galactosan. Wide determination ranges enabled the analysis of samples of different concentration levels. The method showed good precision, both for standard solutions (3.9–5.9% RSD) and for fine particle samples (4.3–8.5% RSD). Co-elution of internal standard (carbon-13-labeled levoglucosan) and sugar alcohols with levoglucosan decreased the sensitivity of levoglucosan determination. The method was used to determine the MA concentrations in ambient fine particle samples from urban background (Helsinki) and rural background (Hyytiälä) in Finland. The average levoglucosan, mannosan, and galactosan concentrations were 77, 8.8, and 4.2 ngm^{-3} in Helsinki (winter 2008–2009) and 17, 2.3, and 1.4 ngm^{-3} in Hyytiälä (spring 2007), respectively. The interrelation of the three MA isomers was fairly constant in the ambient fine particle samples.

Keywords HPAEC/ESI–MS · Validation · Monosaccharide anhydrides · Tracer · Biomass burning · Aerosols

Introduction

Particulate matter (PM) emitted from biomass combustion, such as wildfires, prescribed burns, energy produce, and residential wood burning, can contribute remarkably to the total PM on the global scale. Recently, biomass has become a more and more attractive fuel because it is a renewable energy source and it is CO_2 neutral [1]. Particulate organic (OC) and elemental carbon (EC) emissions from biomass burning have been estimated at 16 Tg year^{-1} [2], which is approximately 34–38% of total carbonaceous aerosol emissions [3]. Residential biomass burning is a minor source globally; however, locally, its contribution to total PM can be substantial [4]. For example, in Helsinki, Finland, the contribution of biomass combustion to OC in PM_1 (particles with aerodynamic diameter smaller than 1 μm) has been 41% during winter while during summer it has been only 3.4% [5]. In Zurich, Switzerland, the contribution of biomass burning to OC in PM_{10} has been ~41% in winter and ~10% in summer and 25±5% and 6±2% to EC, respectively [6]. When biomass combustion takes place, large amounts of numerous organic compounds are produced that alter the chemistry of atmospheric aerosol remarkably. Most of the

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particulate mass originating from biomass burning is concentrated on submicron particles [7]. Particles in the submicron size range affect the radiation budget of earth, directly both scattering and absorbing solar radiation and indirectly via their ability to act as cloud condensation nuclei [8]. The large amount of organic matter concentrated in submicron particles can also cause serious health problems, especially when the concentration of emitted particulate matter is high [9, 10]. It is important to estimate the contribution of particles emitted from biomass combustion to total particulate matter concentrations, as well as to be able to monitor biomass burning episodes. This can be done using source tracer techniques.

Plant biomass is composed mainly of cellulose, hemicelluloses, and lignin, with some extractives and ash components [11–14]. Cellulose contains only anhydrous glucose whereas hemicelluloses contain, besides glucose, many other sugar monomers, for instance, arabinose, galactose, mannose, and xylose [11]. When cellulose and hemicelluloses burn, several organic compounds are produced, including monosaccharide anhydrides (MAs) such as levoglucosan, mannosan, and galactosan [15, 16]. It has been shown that these three MA isomers are produced exclusively as thermal degradation products of plant biomass. Due to their low vapor pressures, MAs are in the atmosphere in particulate phase [17]. Especially, levoglucosan is produced in large amounts in biomass burning processes, and since it is expected to be stable long enough in atmospheric conditions, it has been used as a tracer for biomass burning [12, 18–20]. It has been suggested that levoglucosan cannot be used as a quantitative tracer due to its dependency on combustion condition [21]. Nevertheless, levoglucosan concentrations have been shown to be useful in factor analysis for particulate matter sources [5].

Traditionally, levoglucosan, galactosan, and mannosan have been analyzed from filter samples. In addition to determination of tracer compounds from filter samples, online methods such as aerosol mass spectrometer (AMS) have been used for tracing biomass burning in aerosols. In AMS method, mass-to-charge ratio (m/z) of 60 has been used as a tracer fragment for biomass burning (e.g., [22, 23]). However, because different biomass species contain monosaccharides in different ratios, additional information of the burning material can be achieved by separating the MA isomers with the use of analytical separation methods, such as gas chromatography (GC) or high-performance liquid chromatography (HLPC) coupled to mass spectrometry (MS) (e.g., [24] and references therein; [25–30]). Common for these analytical separation techniques is their good selectivity since different compounds can be separated not only by their chromatographic elution, but also by their m/z. The quantitative analysis is based on monitoring the

molecular ions or their fragmentation products with mass spectrometry. However, not all GC and HPLC methods enable an adequate separation of the monosaccharide anhydride isomers. For isobaric isomers, such as levoglucosan, mannosan, and galactosan, it is important that they are chromatographically well separated because they appear at the same m/z in mass spectra. The disadvantage of especially GC methods is that the sample preparation including derivatization from the original compounds is typically time consuming. Additionally, imperfect derivatization can cause inaccuracy in quantitation. Moreover, these methods often require the use of organic solvents that might be expensive and/or harmful.

The extraction with pure water and the use of high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) have shown to form a simple analytical method for determining MAs in aerosol samples (e.g., [31–35]). Capillary electrophoresis with PAD has also been used for determination of levoglucosan [36]. However, the identification of MAs with PAD is based solely on the chromatographic or electrophoretic separation and comparison to chromatograms and electropherograms of authentic standards. In addition to MAs, atmospheric particles may include other water-soluble compounds, such as sugar alcohols and monosaccharides and disaccharides that may elute simultaneously with target analytes. Therefore, peak identification is conducted more particularly by MS than by PAD.

In this study, we introduce a method that is composed of high-performance anion-exchange chromatography combined to detection with electrospray ionization mass spectrometry (HPAEC/ESI–MS) for the quantitative analysis of levoglucosan, mannosan, and galactosan in atmospheric particulate matter samples. This method combines the advantages of the use of MS and the simple analytical method with HPAEC. The objective was to develop a chromatographic method that separates MA isomers in the shortest feasible analysis run time. We show that the developed method can be routinely used for different kinds of aerosol samples. We also present results of MA measurements from two sites in boreal region: from urban background in Helsinki, Finland, during winter 2008–2009 and from rural background in Hyytiälä, Finland, in spring 2007.

Experimental

Chemicals

MAs, i.e., galactosan (1,6-anhydro-β-D-galactopyranose; Sigma-Aldrich Co., MO, USA), levoglucosan (1,6-anhydro-β-D-glucopyranose; purity 99+%; Acrōs Organics, NJ, USA), and mannosan (1,6-anhydro-β-D-mannopyranose;

purity 98%; Sigma-Aldrich Co), were used for calibration and validation of the HPAEC/ESI–MS method. Carbon-13-labeled levoglucosan (1,6-anhydro-β-D-[UL-$^{13}C_6$]-glucopyranose in dimethyl sulfoxide; 100 μgmL^{-1}, purity 98%, 1.2 mL ampoule; Cambridge Isotope Laboratories, Inc., MA, USA) was used as an internal standard. Monosaccharides and disaccharides, such as D-galactose (purity 98%; Sigma-Aldrich Chemie GmbH, Steinheim, Germany), D-glucose (Sigma Life Science, Sigma-Aldrich Chemie GmbH), D-mannose (Fluka Analytical, Sigma-Aldrich Chemie GmbH), and sucrose (AnalaR, BDH Laboratory Supplies, Poole, England), as well as sugar alcohols, such as D-arabitol (purity 99+%; Sigma, Sigma-Aldrich Chemie GmbH), myo-inositol (purity 99+%; Sigma, Sigma-Aldrich Chemie GmbH), D-mannitol (purity 98+%; Sigma-Aldrich Chemie GmbH), D-sorbitol (purity 98+%; Sigma, Sigma-Aldrich Chemie GmbH), and xylitol (purity 99+%; Sigma, Sigma-Aldrich Chemie GmbH), were used for method development. Deionized water (Milli-Q water; resistivity 18.2 MΩ cm at 25 °C, total organic carbon 3–5 μgL^{-1}) was produced by Millipore Gradient A10 water purification system (Millipore S.A.S., Molsheim, France), and it was used for extraction of analytes and as an eluent.

Standard solutions

The standard solutions of each compound were made by dissolving a weighed amount of a solid standard compound into a weighed amount of deionized water, yielding stock solutions with concentration of about 1.0 mgmL^{-1}. The used balance was Mettler AT200 (Mettler Instrumente AG, Zürich, Switzerland) with readability of 0.1 mg. From stock solutions, diluted standard solutions as well as a mixture of standard compounds were made for the calibration and validation of the HPAEC/ESI–MS method. The content of internal standard ampoule was dissolved in 100 mL of deionized water, yielding internal standard concentration of 1,200 ngmL^{-1}. This stock solution was added both to standard and sample solutions so that its concentration was constantly 120 ngmL^{-1}.

Instrumentation

The analyses were carried out using a Dionex ICS-3000 system designed for ion chromatography analysis coupled to a quadrupole mass spectrometer (Dionex MSQ™). The analytical separation of the analytes was made using a Dionex CarboPac™ PA10 guard column (2 mm i.d.× 50 mm length) and a Dionex CarboPac™ PA10 analytical column (2 mm i.d.×250 mm length), a 2-mm ASRS® 300 suppressor, a CR-ATC anion trap column, and a potassium hydroxide eluent generator (EGC II KOH). Mass spectrometer was used in detection of the MAs, since these compounds do not have response in the conductivity detector that was also coupled to the ICS-3000 system. Analytes were ionized using ESI. For detection, selected ion monitoring mode (SIM) and mass range (m/z 50–350) scanning mode were used. The molecular ions of levoglucosan, mannosan, and galactosan were monitored using SIM mode with m/z 161; the internal standard (carbon-13-labeled levoglucosan) with m/z 167; galactose, glucose, mannose, and inositol with m/z 179; arabitol and xylitol with m/z 151; mannitol and sorbitol with m/z 181; and sucrose with m/z 341. Data were stored and processed using Dionex Chromeleon® (6.8) data system.

Site and sampling conditions

Ambient fine particulate matter (PM$_1$) samples were collected at two sites in boreal region in Finland: at an urban background station in Helsinki (SMEAR III, 60° 12′ N, 24° 58′ E, 30 m above sea level) November 2008–March 2009 and at a forest station in Hyytiälä, Finland (SMEAR II, 61° 51′ N, 24° 17′ E, 179 m above sea level) in March–April 2007. More information about the stations is given by Järvi et al. [37] and by Kulmala et al. [38], respectively. The PM$_1$ filter samples were collected using the four upper stages of a Berner low-pressure impactor [39] prior to the filter sampling to remove particles larger than 1 μm. Filter sampling was conducted in Helsinki by dividing the sample flow and using two filter cassette systems in parallel: one with a polytetrafluoroethylene (PTFE) membrane filter (47 mm, 3.0 μm FS, Fluoropore™, Millipore, Ireland) and the other with two pre-fired quartz filters (47 mm, Tissuquartz™, Pall Life Sciences, MI, USA) one upon the other (front + backup). The sampling duration was typically 10–24 h with exchange usually at 8 a.m. and 6 p.m. Two weekend samplings lasted 44 and 60 h. The flow rate was 40 Lmin^{-1} for each filter. In the rural background in Hyytiälä, a sampler similar to the one used in Helsinki was used, with the exception that the sample flow was not divided in two, and therefore the flow rate was 80 Lmin^{-1} for the filter. The filter material was quartz, and the sampling duration 24 h. All the particulate matter samples were stored at −20 °C prior to analysis.

Sample preparation

A portion of each sample was taken into analysis. From the PTFE filter, a quarter was cut and weighed. From the quartz filter, a 1-cm^2 piece was punched. MAs have been found to be highly water soluble [40, 41], and therefore the extraction of MAs was made simply with water in the present study. Analytes were extracted from a portion of filter into 5.0 mL of deionized water (with internal standard 120 ngmL^{-1}) by a short manual shaking followed by

15 min of gentle rotation. The extract was filtered through an IC Acrodisc® syringe filter (13 mm, 0.45-μm Supor® (PES) membrane, Pall Sciences) that was washed with deionized water prior to filtering. The filtered sample extract was injected into a 50-μL sample loop of the Dionex ICS-3000 system.

Results and discussion

Optimization of analytical conditions

Sample preparation procedure testing

A comparable sample preparation procedure as used in this study has been introduced by Schkolnik et al. [41], with the exception that they had extracted the sample twice with rotation. They reported that the sample preparation procedure had yielded a recovery of 95±3% for levoglucosan. In the present study, the extraction efficiency of the used rotation method was tested by making the extraction twice and analyzing the both extracts separately ($n=5$). In the latter extract, less than 0.6% of each MA was found. Therefore, we decided to use only single extractions for the samples.

The sample preparation procedure was tested for ambient PM_1 samples both on quartz and PTFE filters and compared to the extraction with 30 min of ultrasonic agitation. It was found that for samples on quartz filters no significant difference (2±3%) existed between extractions by rotation and by ultrasonic agitation. For PTFE filters, the corresponding difference was 7±6%. The measured concentrations from PTFE filters ($n=20$) were on average 4%

higher for levoglucosan and 9% for mannosan than from simultaneously sampled quartz filters (Fig. 1a). The measured galactosan concentrations were on average 8% lower from PTFE filters than from quartz filters. Because the results were fairly similar with the two filter materials, the use of water as an extractant seemed to be suitable despite the hydrophobicity of PTFE.

Mass spectrometer conditions

The operating parameters of MS were chosen so that minimum fragmentation and the best sensitivity were achieved. Both positive and negative ionization were tested, and the negative mode gave better results. The cone voltage was tested with −20, −30, −40, −50, −60, −70, and −120 V. The needle voltage was tested with −2, −3, and −4 kV. The largest peak areas of target analytes were achieved when the cone voltage was set to −50 V and the needle voltage to −3 kV. The probe temperature was set to 500 °C in order for water eluent to evaporate completely. The high probe temperature induced a slight dehydration of galactose, glucose, and mannose, forming respective anhydrides (i.e., galactosan, levoglucosan, and mannosan). Therefore, it is important to get the monosaccharides out of the separation column during an analysis run. Otherwise, carryover peaks might exist in the following analysis run, and they might overlap with the peaks of target analytes, causing erroneous determinations.

Chromatographic method

The objective in this study was to develop a chromatographic method that separates MA isomers in the shortest
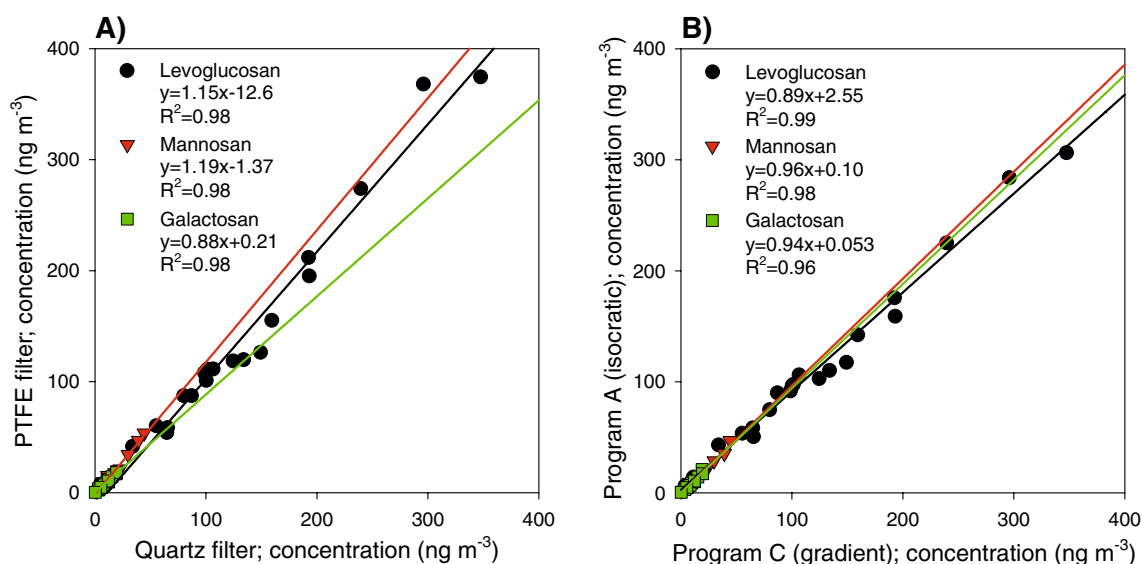
Fig. 1 **a** Comparison of simultaneously collected ambient fine particle samples on quartz and PTFE filters ($n=20$) using program C; **b** comparison of analysis programs with a concentration gradient

analysis (program C) and an isocratic eluent flow (program A) using ambient fine particle samples on quartz filters ($n=20$)

feasible analysis run time. An isocratic program and programs with a concentration gradient followed by an isocratic step with a high eluent concentration for cleaning the column were tested. The best separation between levoglucosan, mannosan, and galactosan was achieved with a low KOH eluent concentration (0.5–2.0 mM) and a low flow rate (0.200–0.250 mLmin^{-1}). These were the lowest conditions possible for the used system, and therefore the analysis run could not be lengthened to get a better separation for the analytes. Using an isocratic program with eluent concentration of 2 mM and flow rate of 0.250 mLmin^{-1} (program A in Table 1), the MA isomers were chromatographically separated and, additionally, monosaccharides and disaccharides as well as some of the sugar alcohols were separated. The isobaric compounds xylitol and arabitol (m/z 151) were not distinguished. Also, sorbitol and mannitol (m/z 181) were poorly distinguished. The run time for eluting all the selected compounds was nearly 25 min. However, the goal was to develop a fast method for routine analysis. In order to accelerate the elution of the compounds after the target analytes (i.e., MAs), a gradient elution program was developed (program B in Table 1). In gradient elution program, the best peak shapes were achieved by the use of a lower flow rate (0.200 mLmin^{-1}) and lower starting eluent concentration (0.5 mM) than in program A. In program B, after the eluent gradient, an isocratic step with a high eluent concentration was added for cleaning out the compounds that have high retention to the stationary phase. With program B, in addition to MAs, some sugar alcohols and monosaccharides and disaccharides could be determined (Fig. 2a). Despite the shorter retention times in program B, the analysis run took time 25 min because of the time needed for conditioning the column back to 0.5 mM after the cleaning step. In our study, the interest was not to analyze monosaccharides and disaccharides but to get them out of the column as quickly as possible. Therefore, the analysis

program could be shortened to 15 min (program C in Table 1) by advancing to the cleaning step, whereupon monosaccharides and disaccharides were incompletely separated (Fig. 2b). The gradient elution programs did not improve the separation of xylitol and arabitol or that of sorbitol and mannitol compared to the isocratic analysis run. The analysis programs with a concentration gradient analysis (program C) and an isocratic eluent flow (program A) were compared using ambient fine particle samples on quartz filters (Fig. 1b). The differences between the determination results achieved using the two programs were not remarkable. The results were on average 10% higher when analyses were conducted using program C than using program A.

Uncertainties of the chromatographic method

The separation between levoglucosan and mannosan was not optimum with the used analytical column, and therefore the determination accuracy of levoglucosan and mannosan was tested with standard additions. The method of standard addition was applied by analyzing unspiked samples and samples spiked with different concentration ratios of levoglucosan and mannosan (10:1, 4:1, 1:1, 1:3). The slopes of the standard additions were good ($R^2 > 0.99$ both for levoglucosan and mannosan, $n = 18$). The difference between the intercept of the standard addition curve and the determination of the unspiked samples was less than 11% for both compounds.

It is known that CarboPac™ PA10 column fails to separate out levoglucosan and mannosan from some sugar alcohols [32]. That was the case here, too: arabitol and xylitol overlapped partly with levoglucosan, whereas sorbitol and mannitol overlapped both with levoglucosan and mannosan (Fig. 2b). The influence of the occurrence of arabitol and mannitol on determination of MAs was tested by adding arabitol and mannitol with three different

Table 1 HPAEC eluent programs and retention times of sugar compounds in standard samples

Program A: Isocratic eluent concentration 2 mM; flow rate 0.250 mLmin^{-1}. Program B: 0.5 mM (1 min)–2.375 mMmin^{-1} (to 10 mM)–4 mMmin^{-1} (to 50 mM)–50 mM (3 min)–0.5 mM (7 min), total run time 25 min; flow rate 0.200 mLmin^{-1}. Program C: 0.5 mM (1 min)–2.375 mMmin^{-1} (to 10 mM)–65 mM (6 min)–0.5 mM (4 min), total run time 15 min; flow rate 0.200 mLmin^{-1}

Analyte	Program A (min)	Program B (min)	Program C (min)
Myo-inositol	2.6	3.2	3.2
Xylitol	2.9	3.6	3.6
D-arabitol	2.9	3.6	3.6
Levoglucosan	3.1	3.8	3.8
D-sorbitol	3.3	4.1	4.1
D-mannitol	3.7	4.5	4.5
Mannosan	3.8	4.7	4.7
Galactosan	5.3	6.4	6.3
D-galactose	16.4	14.6	11.6
D-glucose	19.1	15.6	12.5
D-mannose	24.1	16.4	12.7
Sucrose	19.9	18.4	13.2
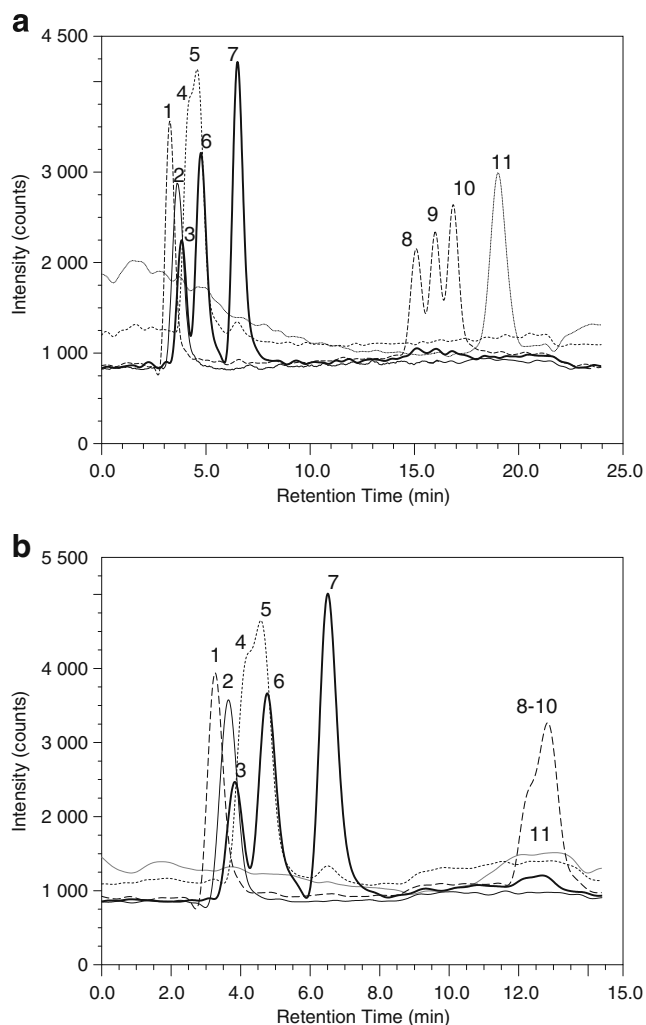
Fig. 2 a Chromatogram of a standard compound mixture with program B. The peaks: *1 myo*-inositol (60 ngmL^{-1}), *2* D-arabitol + xylitol (30 + 30 ngmL^{-1}), *3* levoglucosan (100 ngmL^{-1}), *4* D-sorbitol (30 ngmL^{-1}), *5* D-mannitol (30 ngmL^{-1}), *6* mannosan (100 ngmL^{-1}), *7* galactosan (100 ngmL^{-1}), *8* D-galactose (300 ngmL^{-1}), *9* D-glucose (300 ngmL^{-1}), *10* D-mannose (300 ngmL^{-1}), *11* sucrose (50 ngmL^{-1}). **b** Chromatogram of a standard compound mixture with program C. The peaks: *1 myo*-inositol, *2* D-arabitol + xylitol, *3* levoglucosan, *4* D-sorbitol, *5* D-mannitol, *6* mannosan, *7* galactosan, *8* D-galactose, *9* D-glucose, *10* D-mannose, *11* sucrose. The concentrations were the same as in Fig. 2a

concentration levels (20, 50, and 100 ngmL^{-1}) into the MA standard mixture. To compensate for the ion suppression caused by co-elution of sugar alcohols, the co-elution correction was typically 5–8% of the levoglucosan signal with the highest arabitol and mannitol concentration level. For mannosan, the needed co-elution correction was in the order of 1–2%. The additions did not seem to affect the determination of galactosan. In this study, the concentrations of sugar alcohols in ambient samples were low or non-detectable, and therefore the potential influence of ion suppression caused by those compounds was minor.

Sugar alcohols originate mainly from soil biota, fungi, lichens, and bacteria (e.g., [42–44]), and the active period for those sources is the warm season whereas high concentrations of MAs dominate during the cold season mainly due to domestic usage of wood for heating [42, 45, 46]. Sugar alcohols are known to be mainly associated with the coarse size fraction of forest aerosols [47]. However, remarkable quantities of arabitol and mannitol have also been found in PM$_1$ samples [48]. MAs have been found mainly in fine particles (e.g., [31, 47, 49]).

To take into account the minor influence of sugar alcohols in real cold-season samples, small amounts of sugar alcohols were added to the calibration solutions in the present study. The concentrations of the standard compounds in the stock solution mixture were chosen in that way that they represented the approximate interrelation of the three MA isomers and sugar alcohols in ambient samples in Finland: 200 µgmL^{-1} for levoglucosan, 20 µg mL^{-1} for mannosan and galactosan, and 4 µgmL^{-1} for sugar alcohols.

The concentrations of sugar alcohols and MAs in boreal region aerosols have been presented previously, e.g., from Finland analyzed with GC/MS and HPLC/MS [48, 50] and from Norway with HPLC/high-resolution MS [4, 26, 49, 51]. In the rural site of Hyytiälä in Finland, the concentrations of arabitol (0.0014–0.24 µgm^{-3}) and mannitol (<0.005–0.088 µgm^{-3}) in PM$_{10}$ have been measured in summertime [50]. The highest MA concentrations have been measured in Finland, on the contrary, during the cold season [46]. During the warm season, high concentrations of MAs have been detected in Finland only in special cases, e.g., when smoke plumes have been transported from wild fires [52, 53]. According to Yttri et al. [51], the concentrations of sugar alcohols have been below 0.030 µgm^{-3} in PM$_{10}$, and for PM$_{2.5}$ all concentrations have been below 0.006 µgm^{-3} in Norwegian sites in 2001–2002. At the same time at the same sites, the MA concentrations have been occasionally substantially higher (up to 1 µgm^{-3}), of which 80% were in submicron range [49]. At a Norwegian suburban site, the mean concentration of levoglucosan has been more than 20 times higher in winter than in summer [4].

Analytical performance characteristics

For determining the validation parameters of the HPAEC/ESI–MS method, the purchased standard compounds and ambient fine particle samples were used. In all steps, carbon-13-labeled levoglucosan was used as an internal standard.

Selectivity

The monosaccharide anhydride peaks were detected by monitoring *m/z* 161 and the internal standard by monitoring

m/z 167. The selected ion chromatogram obtained for the mixture of the MA standards is given in Fig. 3a with the mass spectra (m/z 50–350) for all MA peaks (Fig. 3b). Similarly, a chromatogram with mass spectra for all MA peaks of one PM$_1$ filter sample from Helsinki is shown in Fig. 3c, d as an example of all the samples. By means of the figures, it was confirmed that there were no high concentrations of other compounds eluting simultaneously with MAs disturbing the determination, and hence the developed method was considered capable of separating the target compounds from a real ambient sample.

Table 2 presents the chromatographic parameters of monosaccharide anhydrides in aqueous media under the optimized separation conditions. Peak resolution was calculated using standard solution mixture and the equation presented by IUPAC [54]. The peak resolution values depended on the concentrations of the compounds. The best peak resolution was achieved using the lowest concentrations. The separation of levoglucosan and mannosan was not as good as desired since they had baseline separation only with the low concentrations, but they partly overlapped when their concentrations increased. On the linear range, the peak resolution between levoglucosan and mannosan was >1, and with low concentrations it was over 1.5. The peak resolution between mannosan and galactosan was between 2.35 and 3.31, showing good separation throughout the linear range.

Asymmetry values showed that all the three MA peaks were slightly tailing, especially in higher concentrations (Table 2). The peak tailing was not necessarily only due to overload of the separation column, but the analytical column was connected to mass spectrometer via the suppressor, the conductivity detector, and a rather long transfer line that might have caused broadening.

Linearity and range

The linearity and the range of the HPAEC/ESI–MS method were defined by fitting the calibration points (response versus concentration) using linear regression and by calculating the correlation coefficients of the calibration curves using the peak areas of the monitored compounds in relation to the peak area of the internal standard. Each calibration curve contained nine points, and the calibration was made twice on two different days. Calibration for levoglucosan was tested in a concentration range from 1 to 2,000 ngmL^{-1}. The range for mannosan and galactosan was from 1 to 400 ngmL^{-1}. Four replicate injections of standards at each concentration were performed. Calibration curve of levoglucosan showed good linearity in the range of 5–370 ngmL^{-1} with coefficient of determination 0.9995 (Table 2). For mannosan and galactosan, good linearity was shown on a much smaller range. The

quadratic calibration curve showed a good fitting with coefficient of determination higher than 0.999 for each monosaccharide anhydride on a wider range than when linear fitting was used. This enables the analysis of high-concentration samples without an extra dilution of the sample.

Precision and sensitivity

The precision was defined for the standard solutions on eight concentrations and for ambient PM$_1$ samples from Helsinki. The analysis was repeated five times for each sample, and the relative standard deviation (RSD%) of the results was calculated (Table 2). The best precision was obtained for levoglucosan both for standard solution (3.9%) and PM$_1$ sample (4.3%).

The limit of detection (LOD) was defined by using pure standard compounds. The used signal-to-noise (S/N) ratio for LOD was 3. The limit of quantification (LOQ) was defined by using ambient PM$_1$ samples extracted to 5 mL. The used S/N ratio for LOQ was 10. The LOD and LOQ values are given in Table 2. The corresponding limits, reported as needed masses per injection (50 µL) were as follows: LOD for levoglucosan 100 pg, LOD for mannosan and galactosan 50 pg, LOQ for levoglucosan 250 pg, and LOQ for mannosan and galactosan 150 pg. To improve the sensitivity, a smaller extract volume and/or a larger sample size could be used.

Levoglucosan was noticed to have a lower signal on MS than mannosan and galactosan (e.g., Fig. 3a). The higher LOD and LOQ values for levoglucosan than for the two other monosaccharide anhydrides might be due to that reason and, additionally, due to ion suppression that is caused by the co-elution of other compounds (internal standard, possible sugar alcohols in samples) with levoglucosan, which decreases the sensitivity. In order to notice whether other compounds co-elute with target analytes, screening the mass range of m/z 50–350 was used in parallel with the SIM channels. Similar to levoglucosan, internal standard also suffers from ion suppression. However, ion suppression occurs also when calibration is made, and therefore it does not affect the determination of levoglucosan. If the interrelation of MAs in samples changes remarkably, some bias in results of mannosan and galactosan might occur.

Blank measurements were also conducted. No MAs were shown on laboratory blanks, field blanks, or on backup filters of sampled quartz filters.

Accuracy and repeatability

Due to lack of suitable reference material, the accuracy of the HPAEC/ESI–MS method was tested using recovery
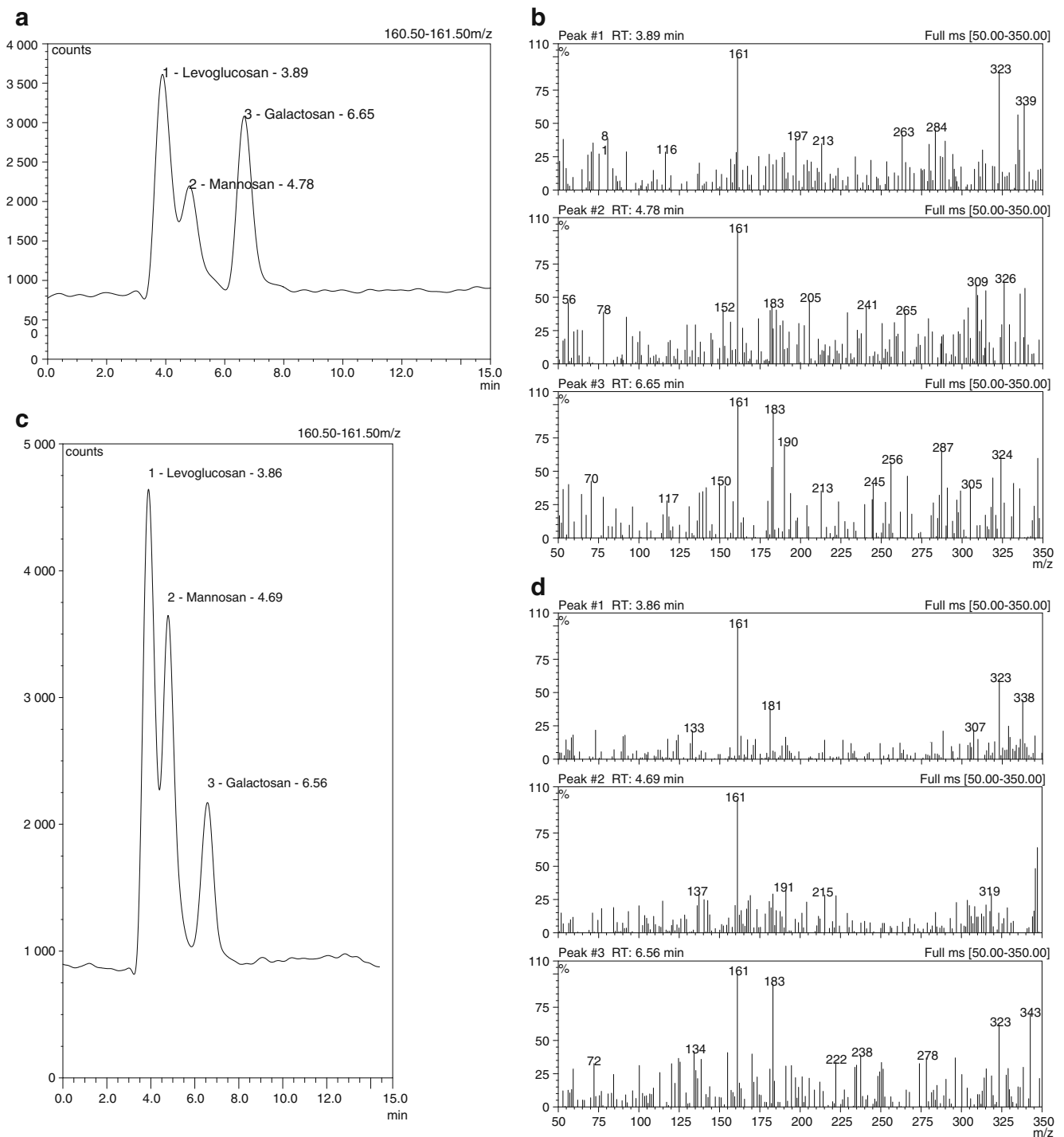
Fig. 3 a Chromatogram of the monosaccharide anhydrides as pure standards in aqueous media. The injection volume was 50 μL, and the standard concentrations were 210, 20.1, and 20.0 ngmL^{-1} for levoglucosan, mannosan, and galactosan, respectively. **b** Mass spectra at the times of MA peaks of the standard mixture presented in **a**. **c** An example chromatogram of the monosaccharide anhydrides in PM$_1$ filter sample from urban background (Helsinki, Finland). The injection volume was 50 μL. The concentrations in sample solution were 228, 29.0, and 12.5 ngmL^{-1} for levoglucosan, mannosan, and galactosan, respectively, and the corresponding ambient concentrations were 0.348, 0.0443, and 0.0191 μgm^{-3}. **d** Mass spectra at times of MA peaks of the ambient PM$_1$ sample presented in Fig. 3c

Table 2 Summary of chromatographic and validation parameters of the HPAEC/ESI–MS method

	Levoglucosan	Mannosan	Galactosan
Peak resolution[a]	1.57–1.04	3.31–2.35	na
Asymmetry[a]	1.13–1.39	1.19–1.32	1.14–1.37
Linear range ($ng mL^{-1}$)	5–370	1–20	1–10
Coefficient of determination in the linear range (%)	99.95	99.63	99.64
Detection limit, LOD ($ng mL^{-1}$)	2	1	1
Quantification limit, LOQ ($ng mL^{-1}$)	5	3	3
Determination range ($ng mL^{-1}$)[b]	5–2,000	3–400	3–400
Precision (% RSD), standard solution	3.9	4.5	5.9
Precision (% RSD), PM_1 sample	4.3	8.5	8.3
Accuracy (%, recovery), quartz filter samples	94±4	103±4	101±6

[a] With the lowest and the highest concentrations in the linear range

[b] Calibration with quadratic curve fitting, coefficient of determination >99.9%

tests. In the recovery tests, known amounts of monosaccharide anhydride standards were added to the sample matrix on quartz filters, and the yield concentrations were determined. The used samples did not contain MAs or contained them only in very low concentrations. The standard additions were made in three concentration levels with three repetitions. The accuracy values (%) are presented in Table 2. The error in accuracy was a bit larger for levoglucosan than for the other two monosaccharide anhydrides.

Repeatability of the HPAEC/ESI–MS method was tested by analyzing MAs from two analogous pieces of five ambient PM_1 filter samples at two different days. The same procedure but different calibrations were used both times. Both times, five co-injections per sample were performed. The repeatability was found to be fairly good. The relative standard deviations within the co-injections were on the range of 1.2–5.0% with an average of 3.3%. The differences of the determinations between the two times were on the range of 1.0–8.5% with an average of 4.8%.

The overall method uncertainty was estimated from validation parameters. The uncertainties (precision) from detection of a standard and detection of a sample and the error in recovery were considered (Table 2). The square root of the sum of the squares of those values was calculated for each MA isomer, producing analytical procedure uncertainties of about 7%, 10.5%, and 12% for levoglucosan, mannosan, and galactosan, respectively. The overall uncertainty of HPAEC/ESI–MS method was calculated by adding the co-elution corrections to the analytical procedure uncertainties. The overall method uncertainties were about 12–15% for each monosaccharide anhydride.

Application of the HPAEC/ESI–MS method for ambient samples

MA concentrations in atmospheric aerosol samples

The HPAEC/ESI–MS method presented in this study has been routinely used for different kinds of aerosol samples.

The smallest mass concentrations of levoglucosan in sampled air that can be quantitatively determined were calculated from the LOQ of the HPAEC/ESI–MS method. The ambient levoglucosan concentration limits for sampling methods used in this study as previously described were 0.0043–0.011 µg m^{-3} for 10–24-h samples (sample volumes 24–58 m^3) collected in Helsinki and 0.0022 µg m^{-3} for 24-h samples (about 115 m^3) from Hyytiälä. The respective concentration limits for mannosan and galactosan were lower due to their lower LOQ than levoglucosan's.

Levoglucosan was quantified from all PM_1 samples from the urban background in Helsinki during winter 2008–2009 (8–19 December 2008 and 14 January–13 March 2009) (Fig. 4a), but mannosan and especially galactosan concentrations were occasionally below LOQ (Fig. 4b). The concentration sum of MA isomers was on average 0.090 µg m^{-3}, of which levoglucosan was 0.077 µg m^{-3} (in the range 0.009–0.384 µg m^{-3}), mannosan was 0.009 µg m^{-3} (below LOQ–0.047 µg m^{-3}), and galactosan was 0.004 µg m^{-3} (below LOQ–0.022 ng m^{-3}). The MA concentrations were on average 12% higher in overnight samples than in daytime samples probably due to low mixing height. Even though the Finnish households are heated with wood mostly in the evenings, no major difference was detected between night and day samples. The atmospheric particles measured in Helsinki during cold season originate primarily from regional sources and only partly from local sources [5]. On average, the highest MA concentrations were determined from the samples that were collected during weekends. The average MA concentration ratio was 1.6 between weekend samples and working day samples. Because the purpose of wood burning is rather pleasure than heating, sauna stoves and fireplaces are heated frequently during weekends when people spend more time at home than on working days.

The MA concentration levels in this study were somewhat lower than previously reported in studies conducted at the same season in Helsinki. Year-to-year differences are related to ambient temperature that is reflected
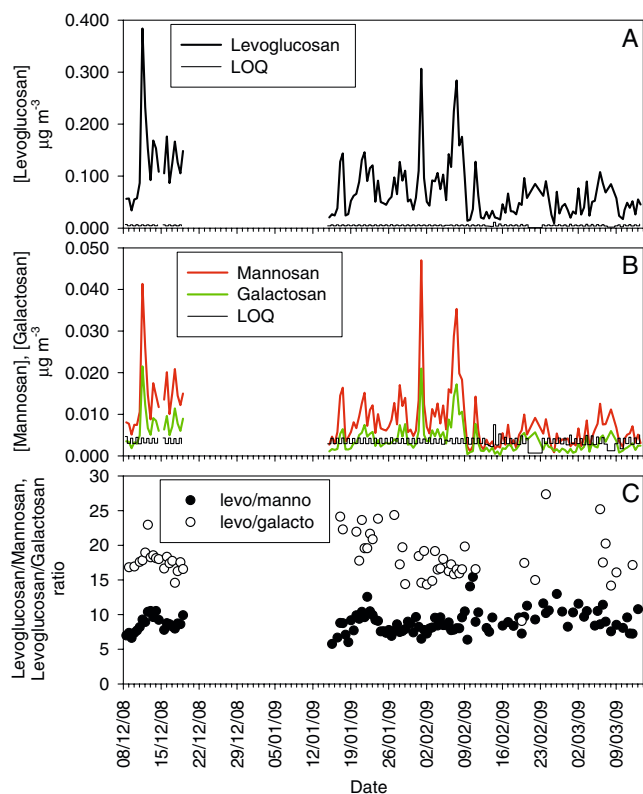
Fig. 4 Ambient concentrations of **a** levoglucosan (*thick black line*), **b** mannosan (*red*), and galactosan (*green*) in PM_1 in Helsinki during December 2008–March 2009. The LOQ concentrations for each sample have been presented with *thin black line*. Ambient concentrations below LOQ have also been presented in the figure; **c** the ratios of levoglucosan to mannosan (levo/manno) and levoglucosan to galactosan (levo/galacto) are presented for concentrations above LOQ

trations in PM_1 were lower than in Helsinki winter 2008–2009. The atmospheric MA in Hyytiälä was caused primarily by regional small-scale wood combustion, but during some days, there were particles either from local sources or from smokes transported from wild fires. During those days, the 24-h averaged MA concentration reached 0.10 $\mu g m^{-3}$. The average MA concentration in spring 2007 was on average 0.020 $\mu g m^{-3}$, of which levoglucosan was 0.017 $\mu g m^{-3}$ (in the range below LOQ–0.079 $\mu g m^{-3}$), mannosan was 0.002 $\mu g m^{-3}$ (below LOQ–0.014 $\mu g m^{-3}$), and galactosan was 0.001 $\mu g m^{-3}$ (below LOQ–0.011 $\mu g m^{-3}$). Typically, the levoglucosan concentration on a filter sample was above LOQ, but the ambient concentrations of mannosan and galactosan were close to LOQ in many samples. The average MA concentration ratio in Hyytiälä was 1.4 between weekend samples and working day samples.

The ambient MA concentration averages that have been reported earlier in the Nordic countries have been higher than the concentrations presented in this study. For example, the average MA concentration has been 0.90 μg m^{-3} in PM_{10} (January–March 2002) in the Swedish small town of Lycksele [21] and 0.53 $\mu g m^{-3}$ in PM_{10} at the Norwegian sites in Oslo (urban background) (November–December 2001) and 0.21 $\mu g m^{-3}$ in PM_{10} in Elverum (suburban) (February–March 2003) [49]. Similar levoglucosan concentrations have been measured typically in cold season also in Central and Southern Europe. For example, the winter (February–March 2003) average concentration for levoglucosan in PM_{10} has been 0.62±0.16 $\mu g m^{-3}$ in urban background in Zurich, Switzerland, while in summer (August–September 2002) it has been 0.07±0.03 $\mu g m^{-3}$ [6]. Puxbaum et al. [33] has presented winter and summer average concentrations of levoglucosan in $PM_{2.5}$ from Central European rural site in K-Puszta, Hungary (0.65 and 0.02 $\mu g m^{-3}$, respectively), and from a coastal semi-urban site in Aveiro, Portugal (1.29 and 0.03 $\mu g m^{-3}$), in 2002–2004.

Interrelation of MA isomers in atmospheric aerosols in Finland

The measured proportions of MA isomers in PM_1 were (average ± standard deviation) 85.8±2.0%/9.7±1.6%/4.5±1.2% (levoglucosan/mannosan/galactosan = L/M/G) during the winter measurements in Helsinki 2008–2009 (*n*=131). The interrelation between the three isomers did not vary significantly during the winter period as can be seen from the uniform alteration of MA isomer concentrations (Fig. 4a, b). The levoglucosan-to-mannosan ratio was on average 8.9±1.6 and levoglucosan-to-galactosan was 18.2±3.2 (Fig. 4c). The levoglucosan-to-mannosan ratio was quite stable but some variation existed in the levoglucosan-to-galactosan ratio because the galactosan concentration

both in emissions and mixing. Higher ambient temperature decreases the need of heating, and at same time the atmospheric mixing is more efficient, causing lower ambient concentrations. Saarikoski et al. [46] has reported a mean MA concentration of 0.25 $\mu g m^{-3}$ in $PM_{2.5}$ during winter 2004 and 0.29 $\mu g m^{-3}$ during spring 2004 at an urban background station in Helsinki. The same concentration level as in the present study has been detected in $PM_{2.5}$ in Helsinki in spring 2003 and summer 2004 when the mean MA concentration has been 0.084 and 0.072 $\mu g m^{-3}$, respectively [46]. Higher warm-season MA concentrations than mentioned above have been detected in PM_1 in Helsinki during long-range transported smoke episodes in spring 2006 [52] and during wild fire smoke plumes in summer 2006 [53]. During these events, the mean levoglucosan concentration has been 0.22 and 0.29 $\mu g m^{-3}$ [52] and 0.24 $\mu g m^{-3}$ [53]. Apart from transported smoke episodes, particulate matter from biomass combustion measured in Helsinki is expected to be mainly regional [5].

In the rural background at Hyytiälä forest station in spring 2007 (28 March–27 April 2007), the MA concen-

was often close to LOQ. No dependence in MA isomer interrelation was detected between the daytime and overnight samples or between the samples on working days and on weekends. At the rural site of Hyytiälä in spring 2007 ($n=$ 33), the MA proportions in PM_1 were $82.5\pm2.7\%/10.9\pm2.7\%/6.6\pm1.9\%$ (L/M/G), and the levoglucosan-to-mannosan ratio was on average 7.1 ± 1.9 and levoglucosan-to-galactosan was 13.0 ± 3.4.

According to the report of the Finnish Forest Research Institute [55], birch has made up a bit more than one third of used firewood in small-sized dwellings in Finland 2007; other broadleaved tree species have made up around one sixth as well as have done both pine and spruce, and the rest has been waste wood. Based on the firewood usage in Finland [55] and the monosaccharide composition in the respective wood species [11], the interrelation of the monosaccharide anhydride isomers in atmospheric aerosol was estimated, assuming that the MAs were produced in a respective interrelationship as the monosaccharides exist in the burnt wood species. In the report of Finnish Forest Research Institute [55], the composition of broadleaved tree species has not been specified. For the estimation of MA proportions produced in burning, aspen was chosen to represent the broadleaved tree species. The portion of waste wood was not taken into account in the estimation. Assuming that glucose produces levoglucosan in pyrolysis, mannose mannosan, and galactose galactosan, respectively, the proportions of produced monosaccharide anhydride isomers from firewood usage in Finland were crudely estimated as 86%/11%/3% (L/M/G). The MA proportions measured in this study did not diverge significantly from the crude emission estimation.

MA isomer interrelation in this study was different to most of those published previously [31, 35, 40, 49]. Engling et al. [35] has presented an extensive review of MA isomer concentrations including several chamber burns and ambient measurements. For example, emission factors for MAs have been presented for different fuel types, such as coniferous tree species (pine and spruce) and different peat types [40]. The MA interrelation closest to the ones of the present study has been reported in bulk aerosols (>1 μm) from Canadian wildfires (83.4%/13.8%/2.8%, L/M/G) by Medeiros et al. [45].

Conclusions

In this study, a quantitative analytical method (HPAEC/ESI–MS) was presented for the determination of monosaccharide anhydrides levoglucosan, mannosan, and galactosan in atmospheric particles. The HPAEC/ESI–MS method is faster and simpler than many of the methods currently available for this purpose. The method is water-based with a simple sample preparation, and it combines fast analytical separation with the advantages of the use of mass spectrometer. The method validation results indicated that, with respect to selectivity, sensitivity, precision, and accuracy, the method showed good applicability in determination of monosaccharide anhydrides. Ion suppression seemed to cause a slight decrease in the sensitivity and accuracy of levoglucosan determination, which is due to the co-elution of the internal standard and possible sugar alcohols in samples.

The new method was applied to the routine analysis of the target compounds in ambient fine particle samples. The HPAEC/ESI–MS method showed good applicability in these determinations. Fast analysis of the monosaccharide anhydrides present in atmospheric fine particles provides valuable information on the quality and sources of ambient particulate matter. In addition, the use of pure water as an extraction agent enables the sample to be used for other water-based analyses, like ion chromatography for determination of inorganic ions and dicarboxylic acids. According to our knowledge, this study was the first application of HPAEC/ESI–MS to monosaccharide anhydrides in atmospheric aerosols.

Findings from ambient results showed that the monosaccharide anhydride isomers exist in ambient aerosol in Finland in certain interrelation that is similar to the one estimated to originate from the Finnish firewood usage. The measured monosaccharide anhydride concentrations were mainly lower than those reported earlier in Finland and in the neighboring countries, which is due to the fact that both emissions and atmospheric mixing are highly dependent on ambient temperature. The highest concentrations were typically found in weekend samples both in urban background and rural background.

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