

Determination of saccharides in atmospheric aerosol using anion-exchange high-performance liquid chromatography and pulsed-amperometric detection

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Abstract

An improved method is described for the quantification of primary sugars, sugar alcohols and anhydrosugars in atmospheric aerosols, making use of separation by high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD). Quartz fibre filters from high-volume samplers were extracted with water and the extract injected directly. Repeatability is typically 4% RSD, for e.g. levoglucosan at 50 ng m^{-3} in air, better for winter levels around 700 ng m^{-3} . Limits of detection for individual sugars are in the range $0.02\text{--}0.05 \mu\text{g mL}^{-1}$ in solution, corresponding to $2\text{--}5 \text{ ng m}^{-3}$ from a 20 m^3 air sample. The overlap of arabinol and levoglucosan is overcome by using a Dionex PA-1 column, with appropriate control of eluent composition, and peak deconvolution software, allowing quantification of both sugars in difficult summer samples containing low-levels of levoglucosan. Analysis of a set of ambient aerosol samples by both GC-flame ionization detection and HPAEC-PAD shows good agreement. The new method has the advantage of requiring no sample pretreatment or derivatization and is thus well suited to handling large numbers of samples.

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1. Introduction

Recent research has revealed that, in addition to numerous organic compounds of low polarity, compounds belonging to various classes of saccharides are important constituents of ambient atmospheric aerosol [1–3]. A practical consequence of these findings is the need to develop specific and precise analytical methods that permit separation and quantification of a range of very polar sugar-type compounds in aerosol samples.

Saccharides present in atmospheric particulates originate from different source types. Micro-organisms, plants and ani-

mals can release into the atmosphere primary saccharides (monosaccharides including glucose, fructose, xylose and disaccharides such as sucrose and trehalose) [2,3], while fungi, lichens and bacteria produce saccharidic polyols, also denoted as sugar alcohols, such as arabinol, mannitol and sorbitol [4,5]. Anhydrosaccharides on the other hand, such as levoglucosan, derived from cellulose, and galactosan and mannosan, derived from hemicelluloses, are the primary thermal degradation products of structural polysaccharides present in biomass [3,6,7].

Primary monosaccharides arise from photosynthetically assimilated carbon in vascular plants, and are an important source of carbon for other living organisms. Some organisms combine two primary monosaccharides into a primary disaccharide for purposes of transport (e.g., sucrose in plants), or as reserve carbohydrate or stress protectant (mycose in bacteria, yeast (unicellular fungi), and a few higher plants as well as insects). Other organisms such as fungi (including lichens, a

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nutritionally specialised symbiotic association of a fungus with a photosynthetic partner) and bacteria reduce monosaccharides to alditols (sugar alcohols) for storage, transport or intracellular osmoregulation. Plants polymerize primary monosaccharides into polysaccharides (e.g., cellulose, a polymer of glucose, and hemicelluloses, polymers of various primary monosaccharides) for growth purposes [8].

Primary biological aerosol particles (PBAPs), comprising pollen, spores, bacteria, fungi, algae, protozoa, viruses and fragments such as vegetative debris, human or animal epithelial cells and parts of insects are significant for the aerosol budget on a global scale, and this applies to all size ranges [9,10]. Bacteria and especially fungal spores contribute at a percentage level to the atmospheric aerosol [11,12] as does plant detritus. This last input can be identified through the amount of cellulose found in atmospheric particulates [13,14]. Simoneit and Elias [15] have linked the composition of the saccharidic mixtures in ambient particulate matter (PM) to the soil biota and proposed resuspension of soil and unpaved road dust as a major component of aerosol particles (the input being either by wind erosion or resuspension from anthropogenic activities) for which saccharides could be used as specific tracers. Table 1 provides a list of important saccharides found in atmospheric aerosols, and their common sources, which is important information in connection with chemical mass balance (CMB) modelling for apportionment of atmospheric aerosol. This paper deals with the development of an HPLC method for the determination of most of the saccharides in Table 1, including levoglucosan, in mixtures from different environmental sources.

Capillary gas chromatography allows the separation of complex mixtures of a large number of compounds, and when coupled with mass spectrometry, of their detection at low concentrations, and often of their identification as well [21]. Compounds of low volatility and/or high polarity will require prior derivatization, such as, for example, into trimethylsilyl ethers or esters, which are volatile enough to be analyzed by GC. It is noted that levoglucosan is sufficiently volatile and leads to a peak in GC/MS, but the peak is broad, difficult to quantify, and can overlap with other compounds [22], making derivatization desirable [2].

Simoneit et al. [7] developed a GC–MS method able to analyze levoglucosan, while Pashynska et al. [1] developed a GC/MS procedure, with derivatization into trimethylsilyl ethers, which permitted the determination of a range of saccharides and achieved satisfactory resolution between levoglucosan, typically a winter species derived from wood smoke, and arabitol, typically a summer species derived from fungal spores and lichens, two compounds which often occur together in the same samples at some times of the year.

When a survey of airborne particulate samples from many sites for many dates has to be carried out, it is tempting to choose methods with the least demand on working and instrument time. Garcia et al. [23] proposed a very fast electrophoresis method for selected saccharides, but the peaks were not sharp and poorly resolved, and only three compounds (levoglucosan, glucose and galactosan) were considered. In addition, HPLC can also be considered because it allows the analysis of under-

Table 1
Saccharides commonly found in atmospheric aerosols and their sources

Compound	Source
Primary sugars (mono- and disaccharides)	
Arabinose	Lichens [4]
Fructose	Lichens [4] Soil biota [2]
Galactose	Soil biota [2]
Glucose	Fungi [16] Lichens [4] Soil biota [2] Wood burning [3]
Mannose	Soil biota [2]
Xylose	Soil biota [2]
Maltose (monohydrated)	Soil biota [2]
Sucrose	Plants [3] Soil biota [2]
Mycose (Trehalose)	Yeast [17] Bacteria, fungi [16] Soil biota [2]
Sugar alcohols	
Arabitol	Fungi, Lichens [4]
Erythritol	Lichens [4] Soil biota [2]
Glycerol	Soil biota [2]
Inositol	Soil biota [2]
Mannitol	Fungal spores [20] Fungi [16] Lichens [4] Soil biota [2]
Sorbitol	Bacteria [18] Lichens [4] Soil biota [2]
Xylitol	Fruits, berries, hardwood [19] Soil biota [2]
Anhydrosugars	
Galactosan (1,6-anhydro- β -D-galactopyranose)	Wood burning [7]
Levoglucosan (1,6-anhydro- β -D-glucose, 1,6-anhydro- β -D-glucopyranose)	Wood burning [6,7]
Mannosan (1,6-anhydro- β -D-mannopyranose)	Wood burning [7]
1,6-Anhydrogluco-furanose	Wood burning [2]

ivatized saccharides in an aqueous extract, hence fulfilling the requirement of faster sample preparation. Two aspects however have to be addressed, namely, the choice of column type and detector.

Reverse-phase HPLC with a C18 column has been evaluated [24] but the resolution of the polar anhydrosaccharides is poor, so that this approach has severe limitations. Ion-exchange systems are generally preferred for carbohydrates, using either cation-exchange in an acidic eluent [25] or anion-exchange in an alkaline eluent [26]. In the acidic system, levoglucosan and mannosan overlap seriously, while in the alkaline system, mannose and glucose overlap, and also levoglucosan and arabitol,

Table 2
HPLC methods reported for the analysis of saccharide mixtures in aerosols

Author, Ref.	Technique	Sugars reported	Samples	Comments
Gao et al. [27]	Electrospray-MS; also HPLC anion-exchange with PAD	Levoglucosan, Mannitol, Glucose, Xylitol, and Glycerol	Smoke	Poor resolution, large uncertainties for xylitol and glycerol
Dixon and Baltzell [28]	HPLC cation-exchange at 80 °C; aerosol charge detection	Levoglucosan, Galactosan, MMP, Mannosan, and Glucose	Atmospheric aerosol	Good resolution, small overlap for Galactosan and MMP
Dye and Yttri [24] Garcia et al. [23]	HPLC C18 column with TOF-MS Microchip electrophoresis pulsed amperometric detection	Anhydrosugars Levoglucosan, Glucose, and Galactosan	Atmospheric aerosol Smoke	Poor separation Poor resolution
Schkolnik et al. [25]	HPLC cation-exchange with UV detection	Mannitol, Arabitol, Erythritol, 2-Methylethritol, 2-Methylthreitol, Levoglucosan, and Mannosan	Smoke-impacted air	Serious overlap between levoglucosan, mannosan and 2-methylthreitol
Engling et al. [26]	HPLC anion-exchange with PAD	Levoglucosan, Mannosan, Galactosan, Mannose, and Glucose	Smoke	Partial overlap of glucose and mannose
Wan and Yu [29]	HPLC anion-exchange with electrospray-MS	Mannitol, Glucose, Levoglucosan, Xylitol, Erythritol, Glycerol, Xylose, Sucrose, and Malezitose	Atmospheric aerosol	Very poor resolution

though the latter shortcoming was not reported by Engling et al. [26]. The present paper describes the results of work done using anion-exchange separation.

Detection by UV would not normally be chosen for carbohydrates because they do not absorb in the easily accessible mid-UV. However, Schkolnik et al. [25] did succeed in using UV detection at 194 nm, though the general experience is that solvents are not transparent enough to be used at this low wavelength. Electrospray ionization (ESI)-MS can offer good sensitivity, but does not provide information about fragmentation for identification purposes, and, moreover, the three anhydrosaccharides, levoglucosan, mannosan and galactosan, are stereoisomers showing the same molecular ion species [24]. In addition, the ESI system requires a completely volatile eluent and hence rules out dilute sodium hydroxide as eluent and the use of anion-exchange separation. For all these reasons, ESI does not appear to be a good choice. Amperometric detection, on the other hand, works very well for carbohydrates, offering excellent sensitivity (especially in pulsed mode) and good selectivity over other classes of compounds. This approach, in which a hydroxyl group in the sugar molecule is oxidized at a gold electrode in the amperometric detection step, has been adopted in the current study.

Most reports on HPLC analysis for saccharides have been directed at smoke emissions, which contain a limited number of saccharidic compounds, and therefore did not have to deal with the difficult overlaps encountered when atmospheric aerosol samples are analyzed. In this paper, we report an HPLC method based on amperometric detection, which permits the quantification, in one run, of five primary monosaccharides (arabinose, galactose, fructose, glucose and mannose), one disaccharide (sucrose), four sugar alcohols (arabitol, mannitol, sorbitol and xylitol) together with the three anhydrosugars galactosan, levoglucosan, and mannosan, not only in wood smoke, but also in ambient atmospheric aerosol samples. The determination by HPAE-PAD has the advantage of omitting the derivatization step, and is thus less time, cost and labor intensive. The

methods proposed recently in the literature are summarized in Table 2.

2. Experimental

2.1. Chemicals

The water used to prepare the standard solutions as well as to extract the samples was $18.2 \text{ M}\Omega \text{ cm}^{-1}$ (Milli-Q, Millipore) water. The following standards were purchased from the suppliers indicated:

levoglucosan: Aldrich 316 555 1G, CAS 498-07-7; galactosan: Sigma-Aldrich A-5180, CAS 644-76-8; mannosan: Sigma-Aldrich A-7429, CAS 14168-65-1; fructose: Sigma-Aldrich F 0127, CAS 57-48-7; galactose: Sigma G 0750, CAS 59-23-4; glucose: Sigma-Aldrich G 8270, CAS 50-99-7; mannose: Sigma 63580, CAS 3458-28-4; sucrose: Merck 8966, CAS 57-50-1; arabitol: Sigma 10880, CAS 488-82-4; mannitol: Merck 5769, CAS 69-65-8; sorbitol: Sigma 85530, CAS 50-70-4; xylitol: Sigma 95649, CAS 87-99-0; methyl β -L-xylanopyranoside: Sigma M-5878; dulcitol: Sigma D0256.

Stock solutions were prepared by dissolving 100 mg of each compound in water and diluting to 100 mL.

For the mobile phase, dilute sodium hydroxide solutions were prepared from a 50% (w/w) NaOH solution (J.T. Baker) using volumetric pipettes. This solution was diluted, 10.5 mL to 1 L, to give a 200 mM solution (B), and 260 μL to 1 L to give a 5 mM solution (C) for the eluent. Care must be taken to minimize exposure of these solutions to atmospheric CO_2 as carbonate will adsorb on the column and lead to deterioration in performance.

Table 3
HPLC eluent programme for the determination of aerosol sugars

Time (min)	Eluent NaOH (mM)	A, Milli-Q water (%)	B, NaOH (5 mM, %)	C, NaOH (200 mM, %)	Sugars separated
0–5	0.5	90	10	–	Xylitol, arabitol, levoglucosan, sorbitol, mannitol, mannosan and galactosan
5–27	20	90	–	10	Arabinose, galactose, glucose, mannose, fructose and sucrose
27–42	200	–	–	100	Cleaning
42–70	0.5	90	10	–	Equilibration

2.2. Apparatus

A Dionex pump model GP-50 was coupled to a Rheodyne 9740 sample injection valve with 10- μ L sample loop, to a Carbopac PA-1 guard column (50 mm \times 4 mm), and then to a Carbopac PA-1 anion-exchange analytical column (25 mm \times 4 mm) and finally to a Dionex model ED 40 electrochemical detector. Between the pump and the autosampler was fitted a GM-3 eluent stream mixer followed by an Ionpac ATC-1 guard column to remove anions from the eluent. The autosampler was a model Basic Marathon from Spark (The Netherlands), and flushed 150 μ L of sample through the 10 μ L sample loop. In-line filters, a 35 μ m filter followed by a 5 μ m filter (in one unit) were placed after the autosampler to remove fine particles from the injected sample.

The three reservoirs on the Dionex GP50 gradient pump were filled with Milli-Q water (A), 5 mM sodium hydroxide (B) and 200 mM sodium hydroxide (C). All solutions were degassed by applying a vacuum to the sonicated eluent for 10 min. After gently pouring the NaOH solution into the reservoirs B and C, all three bottles were purged with helium at approximately 0.5 bar for another 10 min to prevent absorption of CO₂. The programme is summarized in Table 3.

The detector cell has a gold working electrode (P/N 060139) and a pH electrode (P/N 046333) as reference (both from Dionex). A Dionex UCI-50 data acquisition system applies appropriate potentials (see Table 4) to the electrochemical cell and acts as an interface to pass a signal derived from the cell current to the data system using Chromeleon software. A diagram of the complete system is shown in Fig. 1.

2.3. Sample preparation

The filter aliquot to be analyzed (about 4.5 cm² from a quartz fibre filter from a high-volume sampler, corresponding to an air volume of approximately 20 m³, or 0.5–4 μ g of PM₁₀) was

Table 4
Amperometric detector pulse pattern for sugars

Time (s)	Potential (V)	Function
0–0.2	+0.1	Delay
0.2–0.4	+0.1	Integration
0.41–0.43	–2.0	Cleaning
0.43–0.44	+0.6	Conditioning
0.44–0.50	–0.1	Conditioning

extracted in 3 mL of Milli-Q water by ultrasonic bath agitation for 45 min. Undissolved sample material and filter debris were removed from the sample solution by centrifugation (3 min at 13.4 \times 103 rpm) and filtration (35 μ m and 5 μ m, Dionex) prior to injection.

2.4. GC-flame ionization detection (FID) analysis

Arabitol, mannitol and levoglucosan levels were determined for selected samples with an independent GC-FID method using xylitol, dulcitol and methyl β -L-xylanopyranoside as internal recovery standards for arabitol, mannitol and levoglucosan, respectively. Details about the sample workup, the quantitation procedure based on the use of internal recovery standards, and the GC conditions can be found in previous work [1,30,31]. Briefly, a section of 1 cm² of the quartz fibre filter was spiked with methyl β -L-xylanopyranoside (518 ng), xylitol (258 ng) and dulcitol (251 ng), and extracted three times with 20 mL of a mixture of dichloromethane:methanol (80:20, v/v) under ultrasonic agitation. The extract residue was trimethylsilylated with 50 μ L of a 3:5 (v/v) mixture of pyridine and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) containing 1% trimethylchlorosilane (TMCS) (Pierce, Rockford, IL, USA), and directly analyzed by GC-FID.

3. Results and discussion

3.1. Sample extraction

Studies involving a wide range of organic compounds in smoke or atmospheric aerosols, and hence requiring analysis by GC/MS, usually resort to extraction with a dichloromethane–methanol solvent mixture, frequently aided by ultrasonic agitation [1–3]. However, saccharides are all very soluble in water, allowing the use of a much more polar extraction solvent when the analysis is to be carried out by HPLC, which has the additional advantage that derivatization is not necessary. Dye and Yttri [24] used tetrahydrofuran (THF) as an extraction solvent, and then transferred the analytes to water, while both Engling [26] and Schkolnik [25] used only water. In the current study, extraction has been carried out with water, but was aided by ultrasonic agitation.

The extraction step was optimized in terms of ultrasonic agitation time varying between 5 min and 1 h. It was found that extraction times of 45 min lead to a better reproducibility. Recov-

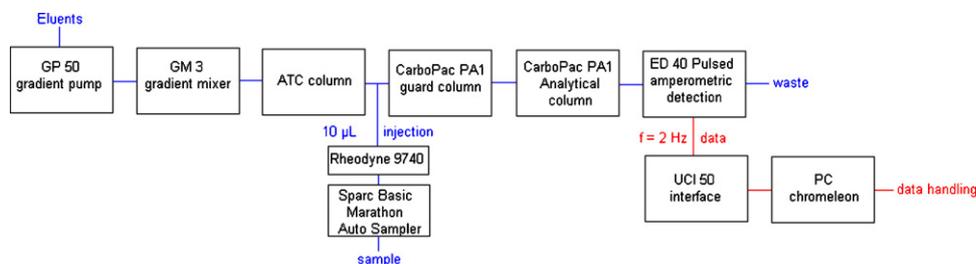


Fig. 1. Scheme of the HPLC setup for determination of sugars in atmospheric aerosols.

eries from quartz fibre filters were in the range $100 \pm 8\%$ when working within the range of the calibration standards.

3.2. Choice of ion-exchange column

The method described in the Dionex Technical Notes 20 and 21 [32] was tested to analyze the sugar content of atmospheric aerosols. The Dionex CarboPac PA-10 was used, as recommended, with a mobile phase of aqueous sodium hydroxide solution increasing from 3.6 to 4.8 mM NaOH over 15 min. However, this method failed to separate some compounds: levoglucosan and arabitol overlapped completely, as did mannitol and mannosan.

Changing to a PA-1 column resulted in a partial separation of these two pairs, and also offered a broader operational pH range. As changing the eluent concentration has consequences for both chromatographic and detector performance, it was necessary first to establish optimum conditions for the detector.

3.3. Detection

Carbohydrates are detected by measuring the electrical current generated by their oxidation at the surface of a gold electrode. In order to overcome problems associated with the removal of oxidation products from the electrode, and to improve the sensitivity, a pattern of changing potentials is applied to the electrode.

In the first step, a voltage is applied between the gold working electrode and the solution, which is high enough to cause electron-transfer reactions thereby oxidizing the carbohydrate molecules adsorbed on the electrode surface. As the application of a potential produces, in addition to the analyte oxidation current, also an initial capacitive charging current, the cell current is measured after a short time delay that allows the charging current to decay. The analyte oxidation current is measured by integrating the cell current (read against an Ag/AgCl electrode) over the integration time. The detector response is then measured in Coulombs (charge = current \times time).

After the integration time, a sequence of two different potentials is used, a first potential is applied to clean the gold working electrode surface from the reaction products, and then a second one to equilibrate the electrode so that the cycle can start again. The equilibration time allows analyte molecules to accumulate under diffusion control, on the electrode surface, thus enhanc-

ing the sensitivity. The waveform requires a total of 500 ms, and data can be collected at 2 Hz.

The measurement potential was varied between 5 and 300 mV (Dionex standard is 100 mV) to evaluate whether a potential could be found at which only one compound would be detected. All compounds of this class showed the same behaviour, implying that one potential can be selected for the detection of all compounds, but that selective detection is not possible (Fig. 2).

3.4. Chromatographic separation

Carbohydrates are very weak acids. At high pH, they are at least partially ionized, and thus can be separated by anion-exchange mechanisms. However, anion-exchange chromatography cannot be performed at high pH with classical silica-based columns, because they are chemically not stable at high pH, but an alkali-stable polymer resin anion-exchange column, such as the Dionex CarboPac PA-1 can be used instead.

Exchange of the different compounds between the eluent and the stationary phase can be controlled by adjustment of the pH, in this case, by changing the sodium hydroxide concentration. While a continuous gradient is often used to achieve separation of complex mixtures, it was found to be more efficient in the current work to use a step programme to elute the two different classes of saccharides.

Sodium hydroxide concentrations between 0.1 and 150 mM were tested on the CarboPac PA-1 column. The separation was better for concentrations in the range of 0.1–0.5 mM, but since the sensitivity of detection by pulsed amperometry for carbohydrates is lower at a lower pH, a concentration of 0.5 mM was

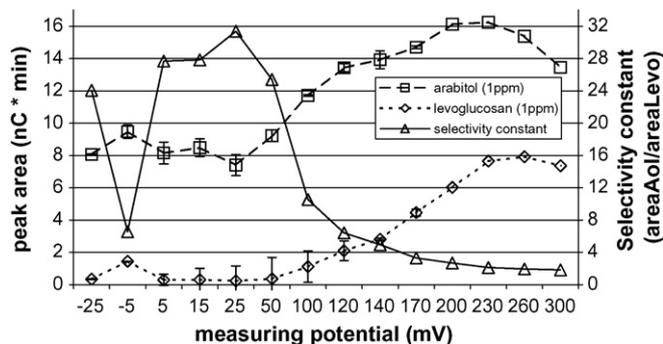


Fig. 2. Detector response as a function of applied measuring potential for arabitol and levoglucosan. The selectivity constant is the ratio between the arabitol and the levoglucosan areas.

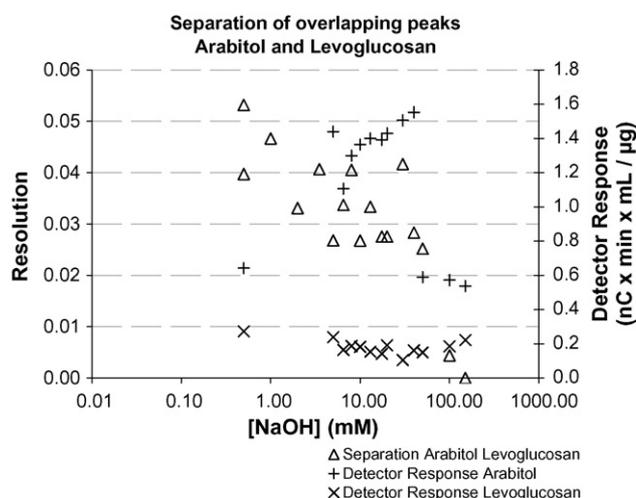


Fig. 3. Peak separation and detector response as a function of eluent composition: Separation = $(T_{\text{levoglucosan}} - T_{\text{arabitol}}) / ((T_{\text{levoglucosan}} + T_{\text{arabitol}}) / 2)$.

selected for the analysis of the sugars alcohols and the anhydrosugars. As too low, a hydroxide concentration is difficult to control, resulting in variable retention times, the lowest practical value was 0.5 mM (Fig. 3). For the primary sugars, which are more strongly retained by the column, the eluent pH could be raised without any loss in selectivity or in sensitivity. The NaOH concentration used for this step was 20 mM.

Addition of methanol to modify the polarity of the eluent was evaluated in an attempt to improve the separation of the difficult pair, arabitol and levoglucosan, but was not successful.

Small improvements in chromatographic performance can also be achieved by adjustment of the flow rate. Fig. 4 shows the variation in column performance, for four of the saccharides, including levoglucosan, as a function of eluent flow rate. The adopted value of 1 mL min^{-1} is probably close to the optimum, since higher flows would require too high an eluent pressure.

3.5. Peak deconvolution

The resulting chromatograms (Fig. 5a and b) show good separations for most compounds, but still a partial overlap can be seen for the peaks of arabitol, usually associated with lichens and

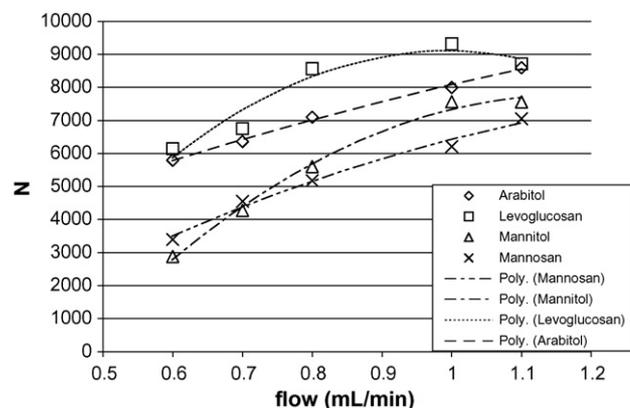


Fig. 4. Column performance (number of theoretical plates, N) for four saccharides as a function of eluent flow rate.

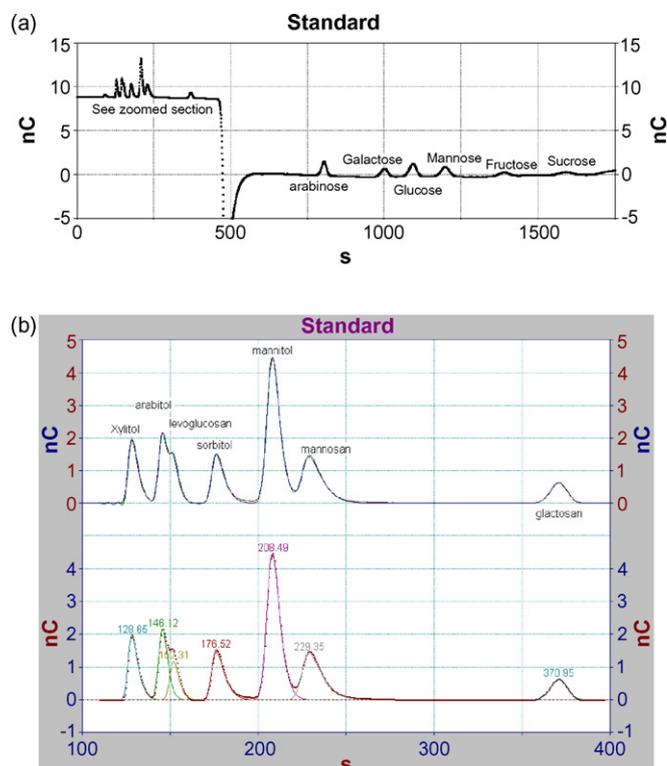


Fig. 5. (a) Chromatogram for the 13-component saccharide mixture: xylitol, arabitol, sorbitol, arabinose, galactose, glucose, mannose, fructose and sucrose: $0.5 \mu\text{g mL}^{-1}$; levoglucosan, mannitol, mannosan: $1 \mu\text{g mL}^{-1}$; galactosan: $0.25 \mu\text{g mL}^{-1}$. (b) Expanded initial section of the chromatogram showing the polyols and the anhydrosugars, plus the peak deconvolution output. The inferior graph shows how the deconvolution software fits the peaks while the above one shows how the fitted data matches the experimental data.

fungal spores, and levoglucosan, accepted as a useful marker for wood smoke. To achieve complete analysis, Peak Fit software (Systat) was used where the shape of the peaks of the different overlapping compounds is expressed in terms of a range of parameters within a type of modified Gaussian curve. The use of a modified Gaussian curve was necessary because the peaks are not symmetrical, but show some tailing, which means that peak fitting with a simple Gaussian peak-fit was not satisfactory, and peak height measurement was simply wrong. The ranges for the different parameters are obtained via the injection of standards that do not contain both of the overlapping compounds (non-overlapping standards). The parameters are checked with standards that contain both the overlapping compounds (“check” standards). If the concentration of the compounds within the “check” standards, calculated with the calibration curve from the non-overlapping standards, is as expected, then we assume the ranges for the parameters are right. Once these ranges for those parameters obtained and validated, one can fit a curve, which respect those parameters, to the experimental data of samples.

The mannitol–mannosan pair was resolved by the eluent pH programme, though because of tailing of the mannitol peak, deconvolution using the peak fit software was also used to obtain more accurate results.

Fig. 6 shows that levoglucosan can be determined precisely in the presence of arabitol by this approach. The circles represent

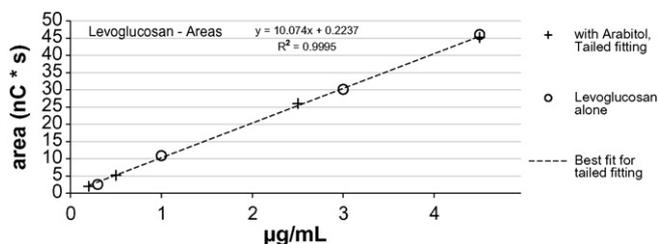


Fig. 6. Levoglucosan calibration with and without arabitol. Common points have the following concentrations (levoglucosan:arabitol in $\mu\text{g mL}^{-1}$): 0.2:0.08; 0.5:0.2; 1:2.5; 2:4.5).

calibration points based on solutions containing only levoglucosan, measured with the deconvolution software, while the crosses show corresponding points for the same levoglucosan concentrations but with arabitol also present, at a comparable concentration of 2 ppm. The dotted line is the best fit for the mixed standard calibration line.

Three sugars listed in Table 1 but not observed in the chromatograms shown in Fig. 5 are maltose, trehalose and xylose, reported to originate from soil [2]. Maltose could be analyzed by adding an additional gradient step to the programme since it elutes later than the other compounds. Xylose elutes between mannose and fructose, while trehalose does between mannosan and galactosan. However, as they were not detected in the selected samples tested for this purpose, their analysis was not included in the method adopted for routine analysis in the AQUELLA (Austrian Aerosol Sources) projects.

3.6. Limits of detection

The limits of detection were assessed as those concentrations giving a peak signal equivalent to three times the standard deviation of the lowest standard, above the blank. Those sugars eluting early in the run, such as xylitol (in step 1) and arabinose (in step 2), gave the sharpest peaks and the lowest l.d. of $0.005 \mu\text{g mL}^{-1}$, while the others were in the range $0.01\text{--}0.03 \mu\text{g mL}^{-1}$. Sorbitol, which gives a broader, tailing peak, showed a l.d. of $0.05 \mu\text{g mL}^{-1}$. The limits of detection are thus determined by the measurement step, and not by blanks or contamination. When applied to filters carrying PM trapped from 720 m^3 of air pumped at 30 L h^{-1} over 24 h, from which normally a section of 1/32 of the whole 150-mm diameter filter is extracted, these limits of detection correspond to 2 ng m^{-3} for mannitol, 3 ng m^{-3} for levoglucosan and 4 ng m^{-3} for arabitol. As levoglucosan levels measured in urban aerosols were typically $40\text{--}50 \text{ ng m}^{-3}$ in summer and $500\text{--}1500 \text{ ng m}^{-3}$ in winter; the method is more than sensitive enough for this purpose.

3.7. Repeatability

A set of standards (13 sugars at five calibration levels in the range $0.1\text{--}5 \mu\text{g mL}^{-1}$ for levoglucosan and $0.04\text{--}2 \mu\text{g mL}^{-1}$ for arabitol) was run in triplicate to assess the measurement performance. To assess procedure performance, five filter pieces, each of 4.5 cm^2 stamped out of one filter from a high-volume sampler, were extracted separately with 3 mL of Milli-Q water

and injected to assess the overall procedure repeatability. One 13.6 cm^2 aliquot was extracted with 9 mL Milli-Q water and this extract was injected five times to assess the measurement repeatability. From the 13 sugars investigated, 6 could be identified in this real sample: arabitol, mannitol, sorbitol, levoglucosan, mannosan, and glucose. The average relative standard deviation was 3.5% for all the sugars except for sorbitol (7%) for which the measured signal was only about 10% of that of the lowest level standard. There was no significant difference in the repeatability between injections of the same extract and injections following different extractions.

3.8. Recovery

Previously baked and equilibrated blank filters (4.5 cm^2 each) were spiked with mixtures of the 13 sugars, extracted and analyzed. In the range $5\text{--}15 \mu\text{g}$ of each sugar (in the 3 mL extract) the recoveries were in the range 95–108%, averaging 102% over ten extractions. At a much lower level of $0.2 \mu\text{g}$ each sugar (around twice the l.d.) the recoveries were still in the range 80–120%. Also, a filter aliquot was extracted three times in order to check for extraction efficiency on a real sample. Between extractions, care was taken to leave as little water as possible inside the vial. The second extraction yielded a peak only for both arabitol and levoglucosan, but below the quantification limit. No peak was observed for the analysis of the third extract.

3.9. Comparison with another method

A comparison was conducted by analysing a number of real samples by the method described here and a GC-FID method developed at the University of Antwerp, for arabitol, levoglucosan and mannitol. Fig. 7 shows the correlation graph for levoglucosan, as an example. Only in two samples out of 23 did the levoglucosan values differ by more than 20%, and only in one sample out of 15 for mannitol, and four out of 15 for arabitol. The average differences were 16, 9 and 15% for arabitol, levoglucosan and mannitol, respectively. Table 5 summarizes the correlation data for these comparisons.

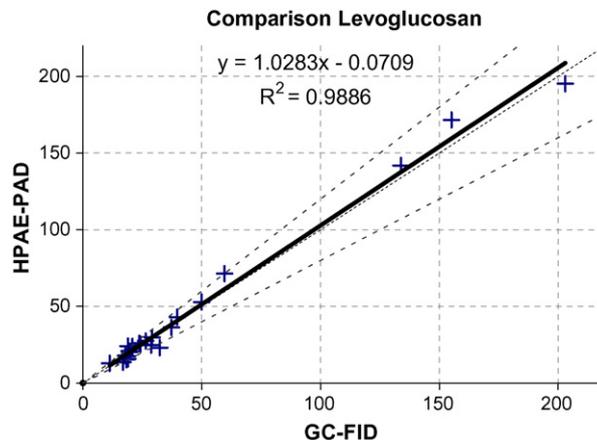


Fig. 7. Correlation between HPLC results and GC-FID results for levoglucosan in a series of atmospheric aerosol samples. The concentrations are in ng m^{-3} in air. Arabitol concentrations in solution were in the range $1\text{--}2 \mu\text{g mL}^{-1}$.

Table 5
Correlation graph data for comparison of techniques (HPLC and GC-FID)

	Arabitol	Levoglucozan	Mannitol
Slope	0.83	1.03	1.03
Correlation R^2	0.96	0.99	0.70

Numbers of samples were 23 for levoglucozan and 15 for arabitol and mannitol. Samples represented various yearly periods and thus concentration ranges were wide: 15–45 $\mu\text{g mL}^{-1}$, 20–80 $\mu\text{g mL}^{-1}$, and 10–200 $\mu\text{g mL}^{-1}$ for arabitol, mannitol and levoglucozan, respectively.

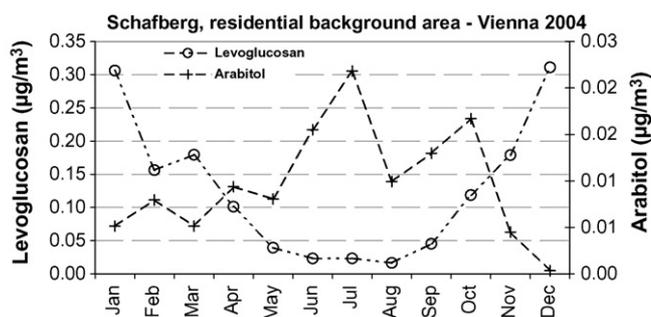


Fig. 8. Variation in arabitol and levoglucozan levels in urban atmospheric aerosols through the year.

3.10. Application to real samples

The new method has been used to analyze large numbers of samples of atmospheric aerosols and of wood smoke as part of a large-scale survey of airborne PM in Austria in 2004 in the frame of the AQUELLA project. For the wood smoke samples it was shown [33] that levoglucozan was present at percentage levels, and can be taken as a quantitative tracer for smoke from traditional domestic stoves. Furthermore, the levoglucozan to mannosan ratio could be used as a good indicator for the ratio of hardwood to softwood being burned.

The relevance of distinguishing levoglucozan and arabitol in atmospheric aerosol samples is demonstrated in Fig. 8, which shows the variation in typical urban particulates through the year, associated with the emission of levoglucozan in wood smoke during winter, and of arabitol from biological sources, in summer.

4. Conclusions

HPAEC-PAD is a very selective method for carbohydrates because: (i) pulsed amperometry detects only those compounds that contain functional groups that are oxidizable at the detection voltage employed (in this case, sensitivity for carbohydrates is orders of magnitude greater than for other classes of analytes); (ii) neutral or cationic sample components in the matrix elute in, or close to, the void volume of the column. Therefore, even if such species are oxidizable, they usually do not interfere with analysis of the saccharidic components of interest. A two-step eluent programme permits the analysis, first, of polyols (arabitol, mannitol, sorbitol and xylitol) and anhydrosugars (galactosan, levoglucozan, and mannosan), and, subsequently, of primary sugars (arabinose, galactose, fructose, glucose, and mannose).

An important problem encountered in the analysis of urban atmospheric aerosols was the overlap of two pairs of compounds, arabitol and levoglucozan, and mannitol and mannosan. In both cases, the compounds belong to different classes and have different aerosol sources. Anion-exchange chromatography using a Dionex Carbopac PA-1 column offered a partial separation of the first pair and virtually complete separation of the second, optimized by careful control of the sodium hydroxide eluent concentration, and final separation was achieved by employing Peak-Fit software to deconvolute the overlapping pairs. This separation allowed quantification of levoglucozan, and of arabitol also as long as the peak height difference was not too high. Thirteen sugars (three anhydrosugars, four sugar polyols, five primary monosaccharides and one primary disaccharide) were included in the method development, six of which are found routinely in urban aerosol samples. Of the six saccharides found in aerosol samples not included in method development, four were tested and did not show overlap. The other two are not expected to occur neither commonly nor in significant quantities. Other possible disaccharides are expected to elute mainly during the cleaning step, at the end of the program, not interfering with the analysis.

The method offers a reliable means of identifying and quantifying the important sugars in simple aqueous extracts of individual portions of quartz fibre filters carrying 0.5–5 μg of particulate matter, collected with high-volume samplers. Levoglucozan and mannosan, both important tracers for wood smoke, could be determined in urban and rural atmospheric aerosol at all times of the year, and at levels well above their detection limits.

An inter-laboratory comparison, in which the proposed HPLC method was tested against a GC-FID method, yielded satisfactory agreement. The use of HPAEC-PAD for the determination of sugars in atmospheric aerosol has the advantage of being both faster and simpler when compared with the GC-based methods which require sample workup and derivatization.

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References

- [1] V. Pashynska, R. Vermeylen, G. Vas, W. Maenhaut, M. Claeys, J. Mass Spectrom. 37 (2002) 1249.
- [2] B.R.T. Simoneit, V.O. Elias, M. Kobayashi, K. Kawamura, A.I. Rushdi, P.M. Medeiros, W.F. Rogge, B.M. Didyk, Environ. Sci. Technol. 38 (2004) 5939.
- [3] P.M. Medeiros, M.H. Conte, J.C. Weber, B.R.T. Simoneit, Atmos. Environ. 40 (2006) 1694.

- [4] L. Dahlman, J. Persson, T. Näsholm, K. Palmqvist, *Planta* 217 (2003) 41.
- [5] B. Graham, P. Guyon, P.E. Taylor, P. Artaxo, W. Maenhaut, M.M. Glovsky, R.C. Flagan, M.O. Andreae, *J. Geophys. Res.* 108 (2003), 6-1.
- [6] F. Shafizadeh, *Adv. Carbohydrate Chem.* 23 (1968) 519.
- [7] B.R.T. Simoneit, J.J. Schauer, C.G. Nolte, D.R. Oros, V.O. Elias, M.P. Fraser, W.F. Rogge, G.R. Cass, *Atmos. Environ.* 33 (1999) 173.
- [8] R.L. Bielsky, in: F.A. Loewus, W. Tanner (Eds.), *Plant Carbohydrates*, vol. 13A, Springer, New York, 1982, p. 158.
- [9] S. Gruber, S. Matthias-Maser, R. Jaenicke, *J. Aerosol Sci.* 28 (1997) S595.
- [10] S. Matthias-Maser, J. Brinkmann, W. Schneider, *Atmos. Environ.* 33 (1999) 3569.
- [11] H. Bauer, A. Kasper-Giebl, F. Zibuschka, R. Hitzenberger, G.F. Kraus, H. Puxbaum, *Anal. Chem.* 74 (2002) 91.
- [12] H. Bauer, A. Kasper-Giebl, M. Löflund, H. Giebl, R. Hitzenberger, F. Zibuschka, H. Puxbaum, *Atmos. Res.* 64 (2002) 109.
- [13] M. Kunit, H. Puxbaum, *Atmos. Environ.* 30 (1996) 1233.
- [14] H. Puxbaum, M. Tenze-Kunit, *Atmos. Environ.* 37 (2003) 3693.
- [15] B.R.T. Simoneit, V.O. Elias, *Mar. Chem.* 69 (2000) 301.
- [16] F. Martin, M. Ramstedt, K. Sönderhäll, D. Canet, *Plant Physiol.* 86 (1988) 935.
- [17] A. Wiemken, *Ant. Leeuwen.* 58 (1990) 209.
- [18] H. Loos, R. Kramer, H. Sahn, G.A. Sprenger, *J. Bacteriol.* 176 (1994) 7688.
- [19] W. Elbert, P.E. Taylor, M.O. Andreae, U. Pöschl, *Atmos. Chem. Phys. Discuss.* 6 (2006) 11317.
- [20] K. Müller, D. van Pinxteren, A. Plewka, B. Svrčina, H. Kramberger, D. Hofmann, K. Bächmann, H. Herrmann, *Atmos. Environ.* 39 (2005) 4219.
- [21] D.R. Oros, B.R.T. Simoneit, *Appl. Geochem.* 16 (2001) 1513.
- [22] T.B. Jordan, A.J. Seen, G.E. Jacobsen, *Atmos. Environ.* 40 (2006) 5316.
- [23] C.D. García, G. Engling, P. Herckes, J.L. Collett Jr., C.S. Henry, *Environ. Sci. Technol.* 39 (2005) 618.
- [24] C. Dye, K.E. Yttri, *Anal. Chem.* 77 (2005) 1853.
- [25] G. Schkolnik, A.H. Falkovich, Y. Rudich, W. Maenhaut, P. Artaxo, *Environ. Sci. Technol.* 39 (2005) 2744.
- [26] G. Engling, C.M. Carrico, S.M. Kreidenweis, J.L. Collett Jr., D.E. Day, W.C. Malm, E. Lincoln, W.M. Hao, Y. Iinuma, H. Hermann, *Atmos. Environ.* 40 (2006) S299.
- [27] S. Gao, D.A. Hegg, P.V. Hobbs, T.W. Kirchstetter, B.I. Magi, M. Sadilek, *J. Geophys. Res. Atmos.* 108 (2003) (SAF 27-1).
- [28] R.W. Dixon, G. Baltzell, *J. Chromatogr. A* 1109 (2006) 214.
- [29] E.C.U. Wan, J.Z. Yu, *J. Chromatogr. A* 1107 (2006) 175.
- [30] Z. Zdráhal, J. Oliveira, R. Vermeylen, M. Claeys, W. Maenhaut, *Environ. Sci. Technol.* 36 (2002) 747.
- [31] A.C. Ion, R. Vermeylen, I. Kourchev, J. Cafmeyer, X. Chi, A. Gelencsér, W. Maenhaut, M. Claeys, *Atmos. Chem. Phys.* 5 (2005) 1805.
- [32] Technical Note 20: analysis of carbohydrates by high performance anion-exchange chromatography with pulsed amperometric detection, Dionex, Sunnyvale, CA, 2000.
- [33] C. Schmidl, et al., *Atmos. Environ.*, doi:10.1016/j.atmosenv.2007.09.028.