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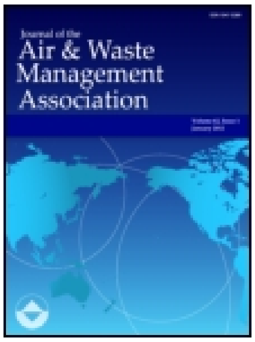

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Determination of Levoglucosan in Atmospheric Fine Particulate Matter

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ABSTRACT

A microanalytical method suitable for the quantitative determination of the sugar anhydride levoglucosan in low-volume samples of atmospheric fine particulate matter (PM) has been developed and validated. The method incorporates two sugar anhydrides as quality control standards. The recovery standard sedoheptulosan (2,7-anhydro-β-D-altro-heptulopyranose) in 20 μL solvent is added onto samples of the atmospheric fine PM and aged for 1 hr before ultrasonic extraction with ethylacetate/triethylamine. The extract is reduced in volume, an internal standard is added (1,5-anhydro-D-mannitol), and a portion of the extract is derivatized with 10% by volume *N*-trimethylsilylimidazole. The derivatized extract is analyzed by gas chromatography/mass spectrometry (GC/MS). The recovery of levoglucosan using this procedure was 69 ± 6% from five filters amended with 2 μg levoglucosan, and the reproducibility of the assay is 9%. The limit of detection is ~0.1 μg/mL, which is equivalent to ~3.5 ng/m^3 for a 10 L/min sampler or ~8.7 ng/m^3 for a 4 L/min personal sampler (assuming 24-hr integrated samples). We demonstrated that levoglucosan concentrations in collocated samples (expressed as ng/m^3) were identical irrespective of whether samples were collected by PM with aerodynamic diameter ≤2.5 μm or PM with aerodynamic diameter ≤10 μm impactors. It was also demonstrated that X-ray fluorescence analysis of samples of atmospheric PM, before levoglucosan determinations, did not alter the levels of levoglucosan.

IMPLICATIONS

Levoglucosan shows promise as a molecular marker for wood smoke. The development and validation of a microanalytical quantitative GC/MS assay suitable for determination of levoglucosan in low-volume samples of ambient atmospheric fine PM is reported here. Use of a chemically similar recovery standard (sedoheptulosan) allows verification of extraction and derivatization efficiency in each sample. The choice of silanizing reagent and conditions is particularly important to ensure quantitative conversion of levoglucosan to the pertrimethylsilyl derivative, before GC/MS analysis. Sensitive, accurate, and reliable methods for measuring levoglucosan in ambient fine particle samples will facilitate the use of this compound in exposure assessment and to apportion wood smoke-derived PM for health studies or regulatory purposes.

INTRODUCTION

Wood smoke emissions from residential fireplaces have been shown to be a major source of particulate matter (PM) in a number of communities in the United States.[1,2] In addition, wood smoke emissions from wildfires and prescribed burns are responsible for occasional severe episodes of air pollution.[3,4] A number of toxic or mutagenic chemicals are present in wood smoke, including polycyclic aromatic hydrocarbons (PAHs), aldehydes, and free radicals.[5–7] Several epidemiologic studies have reported associations between wood smoke exposure and adverse health effects including eye, nose, and throat irritation, decrements in lung function, and increased respiratory infections.[5,8–10] Thus, wood smoke exposure may pose a health concern, especially for sensitive groups, in parts of the United States.

Recent publications have reported analyses of the chemical composition of wood smoke.[7,11,12] These included measurements of fireplace emissions (source samples) and ambient atmospheric PM collected from airsheds impacted by wood burning. Of the hundreds of compounds present in wood smoke, 4-substituted methoxylated phenolic compounds (methoxyphenols) and levoglucosan (a sugar anhydride) have been suggested as potential molecular markers. These compounds are abundant in wood smoke, their presence in atmospheric particles is unique to biomass combustion, and they are relatively stable tracers.[3,12] Reliable molecular markers for wood smoke would facilitate source apportionment studies of atmospheric fine PM and would be useful for studying exposure-effect relationships for wood smoke.

Several authors have previously used gas chromatography/mass spectrometry (GC/MS) methodology to determine levels of levoglucosan in wood smoke and atmospheric PM.[7,12–14] However, comprehensive method validation through the measurement of analyte recovery during extraction, measurement of derivatization efficiency, or the use of appropriate surrogate compounds was typically not reported. These are significant limitations that will affect the accuracy and reliability of the reported levoglucosan measurements. Furthermore, method validation data have not been reported for levoglucosan determination in archived, low-volume ambient PM samples. In this manuscript, the development of a microanalytical, quantitative GC/MS assay for the determination of levoglucosan in atmospheric PM samples is described. Comprehensive details of the assay performance (recovery and precision) are reported, and application of the method to analysis of environmental samples is described.

EXPERIMENTAL METHODS

Chemicals

Levoglucosan (1,6-anhydro-β-D-glucopyranose), sedoheptulosan (2,7-anhydro-β-D-*altro*-heptulopyranose), and 1,5-anhydro-D-mannitol were purchased from Sigma. *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) and *N*-trimethylsilylimidazole (TMSI) were obtained from Supelco. *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) containing 1% trimethylchlorosilane (TMCS) was purchased from Pierce. Ethylacetate (ACS-grade), pyridine (ACS-grade), and triethylamine (HPLC-grade) were purchased from Fisher Scientific.

Aerosol Collections

Atmospheric fine particle samples were collected outside residences in the greater Seattle metropolitan area, Washington. The samples were collected on microporous poly-(tetrafluoroethylene) (PTFE) filter membranes (SKC, Inc.) using Harvard personal environmental monitors (HPEM; 2.5 μm inlet, 4 L/min) and Harvard impactors (2.5 or 10 μm, 10 L/min). All samples were collected for 24 hr.

Sample Preparation

The sample extraction protocol, which utilizes ethylacetate as the extraction solvent, was previously optimized for the determination of methoxyphenols in atmospheric PM.[15] Other solvent mixtures were not investigated for levoglucosan extraction efficiency. The PTFE filter membranes were separated from the polyolefin support rings by using a custom-built Teflon/stainless-steel cutter (see Figure 1), the filter was placed in a 40-mL silane-treated glass vial, and the recovery standard (sedoheptulosan) was amended onto the filter. The vial was sealed with PTFE-faced septa and the amended filter aged for 1 hr. Extraction solvent (30 mL ethylacetate containing 3.6 mM triethylamine) was injected into the headspace vial via a 50-mL glass syringe, and the samples were placed in an ultrasonic bath for 1 hr. After extraction, the solvent was decanted into silane-treated 30-mL concentrator tubes (Zymark Corp.) and reduced in volume to ~0.5 mL at 45 °C and atmospheric pressure, by using a Turbovap concentrator (Zymark Corp.). The extract was filtered through a 0.45-μm, PTFE syringe filter (13 mm diameter; Whatman, Inc.) into silane-treated amber glass autosampler vials, and internal standards (1,5-anhydro-D-mannitol; triisopropylbenzene) were added.

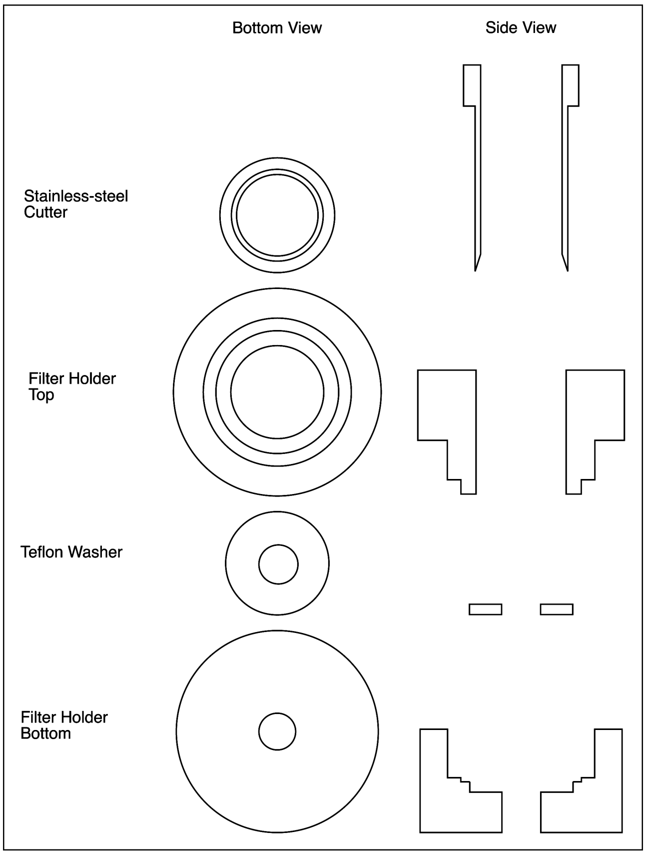

Figure 1. Vertical and horizontal cross-sectional diagram of Teflon/stainless-steel cutting device developed to excise Teflon filter membranes from the polyolefin support ring. The Teflon filter is placed between the two sections of the filter holder for cutting.

Derivatization

An aliquot (100 μL) of the extract was transferred into a 300-μL glass autosampler vial insert. Ten μL of silating reagent (TMSI) was added to the insert, which was then capped and allowed to react at ambient temperature for 1 hr. The extract was subsequently analyzed by GC/MS.

Organic Analysis

The GC/MS system consisted of a CP-8200 autosampler, 1079 temperature programmable injector, CP-3800 gas chromatograph, and Saturn 2000 ion-trap mass spectrometer (Varian, Inc.). The sample (1 μL) was injected split

(1:50) into the injector at 250 °C. The column (RTX-5, Restek; 30 m, 0.25 mm i.d., 0.25 μm film) was held at 55 °C for 1 min, then ramped to 250 °C at 20 °C/min. Helium (He) was used as the carrier gas, at a constant flow of 1 mL/min. The transfer line was maintained at 180 °C, the mass spectrometer manifold at 80 °C, and the ion trap temperature was 150 °C. The range of 150–350 atomic mass units was acquired.

The major fragment ions at *m/z* 204 (levoglucosan, sedoheptulosan), *m/z* 189 (triisopropylbenzene), and *m/z* 217 (1,5-anhydromannitol) were used for quantification. The standard curve for levoglucosan and sedoheptulosan was linear between 0.1 and 50 μg/mL. The limit of detection (signal:noise ratio ~3) was ~0.1 μg/mL, which is equivalent to ~3.5 ng/m^3 for the 10-L/min samplers (~8.7 ng/m^3 for the 4-L/min sampler). The identification of levoglucosan in environmental samples was based on comparison of retention time and mass spectra with the standard compounds.

X-Ray Fluorescence Analysis

Some of the intact filters were subjected to X-ray fluorescence (XRF) analysis before extraction of the filters. XRF analysis was performed by Chester Laboratories as follows. A batch consisting of 12 filters was placed in the XRF spectrometer under vacuum (50 Torr) for 12 hr. During the 12-hr period, each filter was subjected to X-ray bombardment for a total of 45 min, with beam energies of 7.5–55 kV.

RESULTS AND DISCUSSION

Optimization of the Analytical Method

Initial attempts to analyze levoglucosan directly by using GC/MS, without prior derivatization of the analyte, were unsatisfactory. The chromatographic behavior was poor, and the response was lower than that obtainable for the pertrimethylsilyl derivative. Attempts to silylate with BSTFA containing 1% TMCS (10% by volume), either at room temperature or 70 °C for up to 6 hr, gave multiple silylation products in the solvent (ethylacetate). Zdrahal et al.[14] also noted that, in some cases, BSTFA formed multiple derivatization products with levoglucosan. In contrast, other authors report successful derivatization of levoglucosan with BSTFA.[12,13] This apparent contradiction may be caused by differences in the nature of the organic solvent, the volume ratio of solvent to silylation reagent, and the presence or absence of a catalyst or basic co-solvent for the derivatization reaction (e.g., TMCS or pyridine).

Reaction of levoglucosan with MSTFA + TMCS (10% by volume) was slow (relative to TMSI, discussed later), but the addition of pyridine (10% by volume) to the reaction mixture afforded complete reaction within 6 hr at room temperature. The reaction rate did not increase appreciably at 40 °C, compared with room temperature.

Reaction of levoglucosan with TMSI (10% by volume) yielded a single product, with a mass spectrum corresponding to the tri-trimethylsilyl derivative of levoglucosan. The reaction appeared to be complete (no evidence of underivatized or partially derivatized levoglucosan) within 30 min at room temperature. Provided that the extract and the TMSI were well mixed, no degradation of the levoglucosan at 70 °C was observed, and derivatization yield was equivalent at room temperature or after 1 hr at 70 °C. However, if the extract was heated to 70 °C in the absence of TMSI, or the TMSI was not well mixed with the extract before heating, derivatization efficiency was poor. This may be caused by thermal degradation of the underivatized levoglucosan at elevated temperature, as postulated by Poore.[16] Derivatization of sedoheptulosan and 1,5-anhydromannitol by 10% TMSI in ethylacetate was also complete after 1 hr at room temperature.

When the TMSI-treated solutions were introduced into the GC with a 50:1 split ratio, good chromatographic performance was achieved. However, when the same solution was injected splitless, the response for levoglucosan and sedoheptulosan was greatly reduced. In contrast, for the solutions treated with MSTFA, excellent chromatographic performance and improved detection limits (25 ng/mL with signal:noise 5) were obtained using splitless injection. However, the MSTFA reaction appeared to be less robust—several extracts failed to derivatize completely with the MSTFA/TMCS/pyridine mixture but reacted quantitatively with TMSI. The residual acetic acid and water in the ethyl acetate extracts would impair the silylation efficiency of BSTFA or MSTFA to a much greater extent than TMSI; TMSI is compatible with wet samples.[17] Zdrahal et al.[14] used MSTFA with 1% TMCS to consistently form pertrimethylsilated derivative of levoglucosan in PM extracts that had been taken to dryness. Thus, samples were derivatized with TMSI for routine analysis, and MSTFA was used when it was anticipated that greater sensitivity would be required. Representative selected ion chromatograms for analysis of a calibrant solution and an environmental sample are illustrated in Figure 2. No significant deterioration in chromatographic performance or instrumental response was observed over the course of ~250 injections.

The extraction procedure used was previously optimized for determination of methoxyphenols in atmospheric PM.[15] In the present study, the performance of this extraction procedure was investigated for the determination of levoglucosan. To evaluate the recovery of levoglucosan, sedoheptulosan, and 1,5-anhydro-d-mannitol using this procedure, the three sugar anhydrides (2, 0.5, and 0.5 μg) were each amended onto five PTFE

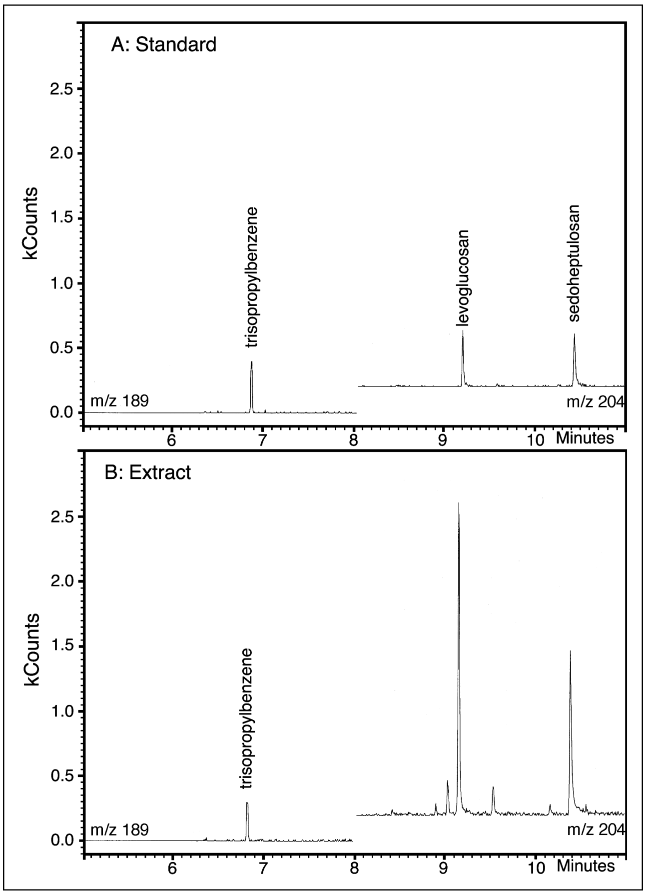

Figure 2. GC/MS selected ion chromatograms for (a) 2.5 μg/mL calibrant mix, and (B) extract of an ambient PM sample.

filters, extracted, derivatized, and analyzed by using GC/MS. Recoveries were 69 ± 6%, 78 ± 7%, and 65 ± 9% for levoglucosan, sedoheptulosan, and 1,5-anhydro-d-mannitol, respectively.

Various filter media, including PTFE, quartz, and Zefluor, are used to collect samples of atmospheric PM. To determine the general applicability of the assay, recoveries of levoglucosan (2 μg) and sedoheptulosan (0.5 μg) amended onto PTFE, quartz, and Zefluor filters were determined. Levoglucosan recoveries (± 1 S.D.) were 90 ± 12%, 76 ± 11%, and 87 ± 18%, and sedoheptulosan recoveries were 100 ± 18%, 25% ± 4%, and 93 ± 20% from PTFE ($n = 4$), quartz ($n = 3$), and Zefluor ($n = 4$) filters, respectively. Therefore, only the recovery of sedoheptulosan from quartz filters was unacceptably low. Sedoheptulosan is not particularly soluble in ethylacetate; thus, the ethylacetate may not be effective at extracting the sedoheptulosan from the quartz filter matrix. The quartz matrix would be expected to sorb sedoheptulosan (via hydrogen bonding interactions) more strongly than the two PTFE-based filter media. In contrast, levoglucosan is readily soluble in ethylacetate at levels at least as high as 1 mg/mL, and good recoveries of levoglucosan were achieved from the quartz filters.

Determination of Levoglucosan in Atmospheric Fine Particles

To evaluate the performance of this assay applied to low-volume environmental samples, nine HPEM samplers were collocated for one 24-hr period. The nine samples were extracted and analyzed as a single analytical batch. The average levoglucosan concentration determined in these samples was 192 ng/m^3, and the reproducibility for these samples was 4.9%. The total $PM_{2.5}$ concentration in these samples was 6.8 ± 0.2 μg/m^3.

We were interested in comparing levoglucosan concentrations in collocated $PM_{2.5}$ and PM_{10} samples. Therefore, archived PM samples collected from 10 pairs of collocated $PM_{2.5}$ and PM_{10} Harvard impactors were analyzed for levoglucosan. The results are summarized in Table 1.

The paired *t* test, when applied to the data in Table 1, indicated that there is no mean difference between levoglucosan concentrations (expressed as ng/m^3) as determined from collocated PM_{10} and $PM_{2.5}$ samplers ($p = 0.768$). This result is consistent with data from Kleeman et al.,[18] who showed that the vast majority of wood smoke particle mass is associated with particles with aerodynamic diameters <1 μm (PM_1). Note, however, that PM mass concentrations were significantly higher for PM_{10} compared with $PM_{2.5}$ (paired *t* test, $p < 0.0001$). Thus, levoglucosan concentrations normalized to PM mass were lower for PM_{10} samples compared with $PM_{2.5}$ samples, and this difference approached statistical significance (paired *t* test, $p = 0.075$). Because the underlying population from which the filter samples were selected may not be normally distributed, the data in Table 1 also were analyzed using a nonparametric method (sign-test). This analysis supported the conclusions drawn from the paired *t* tests.

Often, there is a desire to analyze samples of PM for a variety of different analytes. For example, in ongoing studies, filter samples are subjected to XRF analysis to

Table 1. Concentrations of levoglucosan and ambient particulate (μg/m^3) in collocated $PM_{2.5}$ and PM_{10} samples.

Site No.	PM_{10} Concentration	$PM_{2.5}$ Concentration	Levoglucosan in PM_{10} Sample	Levoglucosan in $PM_{2.5}$ Sample
1	32	26	0.04	0.05
2	40	25	0.03	0.04
3	36	32	0.03	0.03
4	39	26	0.06	0.06
5	35	26	0.01	0.02
6	38	27	0.02	0.02
7	37	25	0.03	0.02
8	30	26	0.60	0.70
9	29	24	0.76	0.70
10	30	23	0.14	0.14

determine concentrations of trace metals. Although XRF analysis of these samples is considered to be nondestructive, the XRF analysis may cause degradation of organic chemical species on the filter, thus compromising subsequent analysis of levoglucosan in the PM samples. Therefore, 10 pairs of PM samples collected with Harvard impactors were selected. One member of each sample pair was subjected to XRF analysis, and then all the filters were analyzed for levoglucosan. The results are summarized in Table 2.

The collocated samplers were either both $PM_{2.5}$ samples (sites 1–3), or collocated PM_{10} and $PM_{2.5}$ samplers (sites 4–10). For sites 4–7, the $PM_{2.5}$ samples received XRF analysis, whereas for sites 8–10, it was the PM_{10} samples that received XRF analysis. Based on the conclusions from Table 1, collocated PM_{10} and $PM_{2.5}$ samples were treated as paired samples for the paired *t* test. There were no significant differences in levoglucosan concentrations (ng/m^3) determined in filters that had been subjected to the XRF analysis when compared with the corresponding collocated sample that was not subjected to XRF analysis (paired *t* test, $p = 0.661$). Thus, it is possible for the PM samples to undergo XRF analysis before analysis for levoglucosan without compromising the levoglucosan measurements. This is in contrast to recent observations that XRF analysis of samples of atmospheric PM caused substantial losses of methoxyphenols.[15]

Table 2. Concentrations of levoglucosan and ambient particulate (μg/m^3) in collocated $PM_{2.5}$ and PM_{10} samplers.

	Sample Subjected to XRF Analysis		Sample Not Subjected to XRF Analysis	
Site No.	PM Concentration	Levoglucosan Concentration	PM Concentration	Levoglucosan Concentration
1	24	0.19	25	0.31
2	21	0.34	21	0.41
3	39	1.65	43	1.53
4	43	1.68	50	1.72
5	41	1.85	52	1.76
6	42	1.64	50	1.76
7	36	1.82	38	1.62
8	35	0.11	22	0.13
9	27	0.47	22	0.53
10	27	0.60	21	0.74

The samples analyzed in Tables 1 and 2 were selected from an underlying population of filters collected as part of an ongoing panel study in Seattle. This population includes samples collected at a central urban site and residential outdoor and residential indoor samples. The samples analyzed for this manuscript covered a wide range of levoglucosan concentrations and represent the three different types of sample locations in the larger study.

CONLUSIONS

A method for the quantitative determination of levoglucosan in atmospheric fine PM has been developed. The method incorporates an anhydrosugar recovery standard and employs GC/MS analysis of the anhydrosugars after trimethylsilation. Acceptable reproducibility and precision for determination of levoglucosan in collocated environmental samples collected on PTFE membrane filters, and for levoglucosan amended onto PTFE, quartz, and Zefluor membrane filters, was determined. It was also demonstrated that levoglucosan concentrations, expressed as ng/m^3, were identical in PM samples collected by collocated PM_{10} and $PM_{2.5}$ samplers, and XRF analysis of samples before levoglucosan determination did not compromise levoglucosan measurement. The use of a chemically similar recovery (surrogate) standard to monitor extraction and derivatization efficiency is an important feature of this assay. Sedoheptulosan proved to be an acceptable surrogate compound when the assay was applied to samples collected on PTFE filters. However, the poor recovery of sedoheptulosan from quartz filters and the differential solubility of the two sugars in nonpolar organic solvents indicate that sedoheptulosan is not an ideal surrogate for levoglucosan. Synthesis of an appropriate isotopically labeled levoglucosan surrogate should, therefore, be a priority.

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