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Determination of Methoxyphenols in Ambient Atmospheric Particulate Matter: Tracers for Wood Combustion

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Combustion of wood and other biomass fuels produces source-specific organic compounds arising from pyrolysis of lignin, including substantial amounts of 4-substituted methoxylated phenolic compounds (methoxyphenols). These compounds have been used as atmospheric markers to determine the contribution of wood smoke to ambient atmospheric fine particulate matter (PM). However, reliable quantification of methoxyphenols represents an analytical challenge because these compounds are polar, semi-volatile, and somewhat reactive. We report herein an improved gas chromatographic−mass spectrometric (GC/MS) method for the sensitive and reliable determination of methoxyphenols in low-volume ambient PM samples. Deuterated standard compounds are added to the environmental samples prior to extraction to determine analyte recoveries in each sample. Analytical figures of merit for the assay, as applied to ambient $PM_{2.5}$ and PM_{10} samples are as follows: recovery = 63−100%; precision = 2−6%; analytical limit of detection (S/N 2) = 0.002 μg/mL; limit of quantitation = 0.07−0.45 ng/m^3 (assuming a 14 m^3 sample). The improved method was applied to ambient PM samples collected between 1999 and 2000 in Seattle, WA. Particle-bound methoxyphenol concentrations in the range <0.1 to 22 ng/m^3 were observed and the methoxyphenols were present almost exclusively in the fine ($PM_{2.5}$) size fraction. We also demonstrated that XRF analysis of samples of atmospheric PM collected on Teflon filters significantly reduced the levels of methoxyphenols measured in the PM samples in subsequent assay of the same filters. Therefore, XRF analysis of filters, commonly undertaken to obtain trace element concentrations for use in source apportionment analyses, would preclude the subsequent analysis of those filters for methoxyphenols and other similarly semivolatile or reactive organic chemicals.

Introduction

Wood smoke emissions from residential fireplaces are a major source of atmospheric fine particulate matter (PM) in several communities in the United States (*1*−*3*). In addition, wood smoke emissions from wildfires and prescribed burns are responsible for occasional severe episodes of air pollution (*4*−*7*). In the developing world, biomass fuels are still the major source of energy for cooking and space heating, and indoor exposures of up to several mg/m^3 have been reported (*4*, *8*, *9*).

Several toxic or mutagenic chemicals are present in wood smoke, including polycyclic aromatic hydrocarbons, aldehydes, and free radicals (*10*−*12*). Several epidemiological 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 (*4*, *8*, *9*, *13*). Children with asthma appear to be particularly susceptible to the effects of wood smoke; reported symptoms included increased respiratory symptoms, lower respiratory infections, and decreased pulmonary function (*13*).

A wealth of recent publications have reported on the chemical composition of wood smoke (*12*, *14*−*16*) from source samples. Of the hundreds of organic compounds present in wood smoke, 4-substituted methoxylated phenolic compounds (methoxyphenols) and levoglucosan (a sugar anhydride) have been suggested as potential molecular markers (*3*, *17*). Methoxyphenols are abundant in wood smoke, their presence in atmospheric PM is unique to biomass combustion, and they are relatively stable tracers. Reliable molecular markers for wood smoke would facilitate source apportionment studies of atmospheric PM and would be useful for studying exposure−health effect relationships for wood smoke. Emission rates for total methoxyphenols were in the range 900−4200 mg/kg fuel, and the compounds were present both in the vapor phase and bound to particles (*12*, *14*, *15*).

In contrast, relatively few reports exist describing methoxyphenol levels in ambient PM samples. Hawthorne et al. reported total methoxyphenol levels in the range 354−3510 ng/m^3 (median = 907 ng/m^3) in ambient samples collected in wintertime 1988−1989 in Minneapolis, MN, and Salt Lake City, UT (*17*). They used a high-volume PM_{10} sampler containing a quartz filter backed with polyurethane foam cartridges; thus, their measurements represent a sum of both particle-bound and vapor-phase methoxyphenols. Schauer et al. described 1995 wintertime methoxyphenol levels at three locations in the San Joaquin Valley, CA: Fresno, Bakersfield, and the Kern Wildlife Refuge. The sum of methoxyphenols ranged from 0.4 to 876 ng/m^3 (median = 97 ng/m^3) (*3*), with the highest concentrations occurring during a wood smoke-dominated, relatively severe air pollution event in Fresno (fine PM levels = 55 μg/m^3). The samples were collected on quartz filters using a high-volume (10 m^3/h) dichotomous virtual impactor and represent only the particle-bound methoxyphenols.

Previous measurements of methoxyphenols in wood smoke and ambient PM have used GC/MS methodology (*3*, *14*, *15*, *18*, *19*). However, we discovered several important analytical issues related to these methods. Many of these studies did not employ authentic standard compounds to calibrate the GC retention times and the MS detector response, and stable-isotope-labeled compounds were not used to validate analyte recoveries. In seeking to validate a sensitive and reliable analytical method for the determination of particle-associated methoxyphenols in ambient PM samples, we encountered unexpected problems including chemical reactivity and volatilization of the methoxyphenols. We describe herein an improved quantitative GC/MS assay for the determination of particle-bound methoxyphenols in low-volume ambient PM samples, in which multiple iso-

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TABLE 1. Purity and Deuteration Conditions for Synthesis of Recovery Standards

chemical	deuteration reaction temp (°C)	deuteration reaction time (h)	chemical purity (%)	isotopic purity (%)
d_6-acetovanillone	150	14.9	100	51[a]
d_2-4-methylsyringol	150	9.9	91	97[b]
d_2-4-ethylsyringol	150	9.9	99	91[b]
d_2-4-propylsyringol	150	6	97	92[b]

[a] 40% d_7 and 9% d_5. [b] Balance was d_1.

topically labeled surrogate compounds are used to monitor analyte recovery.

Experimental Section

Chemicals. 2,3-Dimethoxyphenol, acetovanillone, guaiacylacetone (4-hydroxy-3-methoxyphenylacetone), coniferylaldehyde (4-hydroxy-3-methoxycinnamaldehyde), sinapylaldehdye (*trans*-3,5-dimethoxy-4-hydroxycinnamaldehdye), acetosyringone (3′,5′-dimethoxy-4′-hydroxyacetophenone), trimethoxybenzene, 2-chloro-4-methoxyphenol, and 2,6-dimethoxyphenol were purchased from Aldrich (Milwaukee, WI). 4-Ethylguaiacol and 4-propylguaiacol were obtained from Lancaster Synthesis (Windham, NH). Ethyl acetate (ACS grade) and triethylamine (HPLC grade) were purchased from Fisher Scientific.

Chemical Synthesis. Eight deuterated methoxyphenols were prepared as described in our previous study (*20*). Four additional deuterated compounds, listed in Table 1, were prepared from the proteo-analogue by deuterium exchange (D_2O) under acid conditions (DCl and $CrCl_2$) (*20*). Ring protons were exchanged during the reaction except for acetovanillone, which appears to have also exchanged side chain protons. Hydroxyl deuterium was exchanged back to hydrogen during workup. Compounds were purified by silica gel chromatography using dichloromethane as eluent. Table 1 gives reaction conditions and purities. Isotopic purity was determined from the isotope ratios of the molecular ion cluster. Chemical purity was assessed by gas chromatography with flame ionization detection.

Hydrogenation (5% palladium on charcoal) of syringaldehyde and acetosyringone gave 4-methylsyringol (mp 39.5−41°, lit. mp 40.5−41.5; purity 99.2%) and 4-ethylsyringol (purity 91.7%), respectively (*21*). 4-Allylsyringol was synthesized from syringol and allyl bromide via the ether, 1-allylsyringol (*21*), and was purified by fractional vacuum distillation followed by recrystallization from methanol (mp 28−31°; purity 99.1%). 4-*n*-Propylsyringol was made by hydrogenation of 4-allylsyringol (*21*) and purified by vacuum distillation (purity 99.9%).

Propionylsyringol (propylsyringone; 1-(4-hydroxy-3,5-dimethoxyphenyl)-propan-1-one) and butyrylsyringol (butylsyringone; 1-(4-hydroxy-3,5-dimethoxyphenyl)-butan-1-one) were synthesized by condensing syringol and the corresponding carboxylic acid in the presence of BF_3 (*22*). Syringol (32.4 mmol) and the acid (propionic or butyric; 25.9 mmol) were heated with stirring to 65−85 °C under nitrogen. BF_3 was slowly bubbled through the solution to saturate it. The black-brown solution was stirred for 1.5 h, removed from the heat, and kept at room temperature overnight. Equal volumes of diethyl ether and water (50 mL) were added. The ether layer was separated, washed twice with 100 mL water, twice with 100 mL 5% $NaHCO_3$, and finally three times with 80 mL 5% NaOH. The NaOH solution was made acidic with concentrated HCl and extracted three times with 200 mL dichloromethane. The dichloromethane was dried with sodium sulfate and then evaporated at reduced pressure. The residue (syringol and the syringone) was separated on a silica column (dichloromethane). The syringones were recrystallized from methanol−water. Butylsyringone: purity 93.5%; mass spectra: m/z (%)181 (100), 224 (24), 153 (11), 196 (8); mp 80−82°. Propylsyringone: purity 99.1%; mass spectra: m/z (%) 181 (100), 210 (28), 153 (18), 123 (5); mp 113−115°.

Acetates of each analyte were synthesized using the procedure of Chau and Coburn (*23*) with some modification; three to four washes with 5% HCl were necessary to remove pyridine from the product. Column chromatography (silica with dichloromethane) was used to purify acetates, whose purity after isolation was >97%.

Sinapylaldehyde propionate was synthesized analogously to the acetates but using propionic anhydride. The compound was recrystallized from toluene (mp 132°; purity 99.6% trans, 0.4% cis; mass spectra: m/z (%) 208 (100), 177 (38), 180 (32), 165 (27), 137 (14)).

Aerosol Collections. This study is part of the larger Seattle, WA, exposure and health effect panel study (*24*). This study used 24-h integrated samples collected from subjects' residences and one centrally located outdoor site between Oct 25, 1999 and January 2001. PM_{10} and $PM_{2.5}$ samples were collected over 24 h (4 PM−4 PM) using the single-stage inertial Harvard Impactors (HI) (Air Diagnostics and Engineering, Inc., Naples, ME) at 10 LPM and 37-mm Teflon filters (*25*). One pair of $HI_{2.5}$ and HI_{10} was located in the main activity room of each subject's home, while a second pair was located outside the home concurrently. The limit of detection and precision for the 24-h integrated HI is 1 and 1.2 $\mu g/m^3$, respectively (*24*). Two particulate matter reference materials, SRM 1649a (urban dust) and SRM 2975 (diesel PM), available from NIST (Gaithersburg, MD), were also used in method development studies.

Sample Preparation. All filters were equilibrated at 35% relative humidity and 22 °C for at least 24 h prior to weighing (*26*). All filter weights were measured in either duplicate or triplicate using a seven-place electronic ultramicrobalance (Mettler Toledo, Model UMT2, Greifensee, Switzerland). After weighing, filters were stored at −4 °C until further chemical analysis. All glassware coming in contact with the sample or sample extracts was silylated with hexamethyldisilazane prior to use by a vapor-phase method (*27*). Prior to extraction, the polyolefin support ring must be separated from the Teflon filter membrane. This is necessary because chemical contaminants are present in the support ring that interfere with subsequent GC/MS analyses. The Teflon filter membranes were separated from the polyolefin support ring by using a custom-built Teflon/stainless steel cutter, described in detail in an earlier report (*28*). The filter was placed in a 40-mL glass headspace vial and deuterated recovery standards were spiked onto the filter (~40 ng/compound in 20 μL ethyl acetate). The vial was sealed and the spike allowed to age for 1 h. Extraction solvent (30 mL ethyl acetate containing 3.6 mM triethylamine) was injected into the headspace vial through a Teflon-faced silicone septa, and the samples were then placed in an ultrasonic bath at ambient temperature for 1 h. After sonication, the solvent was decanted into 50-mL Turbovap tubes and was reduced in volume under nitrogen to approximately 0.5 mL at 45 °C and atmospheric pressure. Extracts were filtered through 0.45-μm PTFE syringe filters, into amber glass autosampler vials, and internal standard mix (2,3-dimethoxyphenol, trimethoxybenzene, and 2-chloro-4-methoxyphenol, 0.32 μg in 20 μL ethyl acetate) was added.

Samples of the PM reference materials were prepared and extracted as described for the filter samples, with the following exceptions. An aliquot of the PM reference material was weighed into a centrifuge tube. After the PM sample had been extracted, it was centrifuged at 1800*g* for 30 min, and the supernatant was decanted into the Turbovap tubes.

TABLE 2. Methoxyphenol Analytes, Quantitation Ions, and Retention Times

compd no.	compd type[a]	compd name	retention time (min)[b]	quantitation ion
1	ISTD	trimethoxybenzene	7.43	168
2	rec. std	d_4-guaiacol	6.92	113
3	analyte	guaiacol	6.93	109
4	rec. std	d_3-methylguaiacol	7.91	126
5	analyte	methylguaiacol	7.91	123
6	ISTD	2,3-dimethoxyphenol	8.48	154
7	rec. std	d_3-ethylguaiacol	8.68	140
8	analyte	ethylguaiacol	8.68	137
9	ISTD	2-chloro-4-methoxyphenol	8.71	143
10	rec. std	d_3-syringol	8.90	157
11	analyte	syringol	8.91	154
12	rec. std	d_3-eugenol	9.34	167
13	analyte	eugenol	9.34	164
14	rec. std	d_3-propylguaiacol	9.41	140
15	analyte	propylguaiacol	9.42	137
16	rec. std	d_2-vanillin	9.44	154
17	analyte	vanillin	9.44	151
18	analyte	*cis*-isoeugenol	9.70	164
19	rec. std	d_2-methylsyringol	9.70	170
20	analyte	methylsyringol	9.70	168
21	analyte	*trans*-isoeugenol	10.13	164
22	rec. std	d_6-acetovanillone	10.23	154
23	analyte	acetovanillone	10.26	151
24	rec. std	d_2-ethylsyringol	10.30	169
25	analyte	ethylsyringol	10.30	167
26	analyte	guaiacylacetone	10.76	137
27	analyte	allylsyringol	10.86	194
28	rec. std	d_2-propylsyringol	10.91	169
29	analyte	propylsyringol	10.91	167
30	rec. std	d_2-syringaldehyde	11.00	184
31	analyte	syringaldehyde	11.00	182
32	analyte	acetosyringone	11.63	181
33	analyte	coniferylaldehyde	12.06	178
34	analyte	propionylsyringol	12.27	181
35	analyte	butyrylsyringol	12.81	181
36	ISTD	sinapylaldehyde	13.31	208
37	analyte	sinapylaldehyde propionate	13.90	208

[a] ISTD, internal standard; rec. std, recovery standard. [b] These data pertain to the acetate derivatives of the methoxyphenols.

We compared assay performance for GC/MS analysis of the methoxyphenols as free phenols and after conversion to their acetate derivatives. To form the acetate derivatives, the extracts were treated with 50 μL of a 4:3 solution of acetic anhydride in triethylamine, the vial was capped, and then it was heated at 70 °C for 3 h.

Organic Analysis. The extracts were analyzed for methoxyphenols by using GC/MS with selected ion monitoring (SIM). The GC/MS system consisted of an HP-7673 autosampler, HP5890 series II gas chromatograph, and HP 5989A MS engine mass spectrometer (Agilent Technologies, Wilmington, DE). The sample (2 μL) was injected splitless into the injector at 250 °C. The column (RTX-5 Sil-MS, Restek, Bellefonte, PA; 30 m, 0.25 mm i.d., 0.25-μm film) was held at 55 °C for 1 min, then ramped to 280 °C at 15 °C/min. Helium was used as the carrier gas, at a constant pressure of 18 psi. The transfer line was maintained at 280 °C, the mass spectrometer source at 280 °C, and the manifold at 110 °C. The mass spectrometer was operated in EI mode, with an ionization energy of 70 eV.

The major fragment ions listed in Table 2 were used for quantification. To enhance sensitivity, these ions were acquired in six time-programmed acquisition windows, with a maximum of eight specific ions per window. The standard curve for the methoxyphenols was linear between 0.01 μg/mL and 2.0 μg/mL. The analytical limit of detection (S/N = 2) was 0.002 μg/mL. Low and variable amounts of some analytes were detected in blank filter extracts, in the range 0.002−0.013 μg/mL. Therefore, we defined the limit of quantitation for each analyte as the greater of the average concentration of analyte detected in blank filter extracts, or the analytical limit of detection. Methoxyphenols in environmental samples were identified by the presence of a chromatographic peak with the expected m/z ratio and comparison of retention time relative to the appropriate labeled standard compound. The relative retention time data were obtained from the calibrants. This approach accounted for systematic shifts in absolute retention time between samples and calibrant solutions and facilitated more reliable analyte identification in the environmental samples.

Selected filter extracts were also analyzed for levoglucosan, as described previously (*28*). The levoglucosan was converted to a pertrimethylsilyl derivative, followed by GC/MS analysis.

Results and Discussion

Initially, we evaluated recoveries of a mixture of deuterium-labeled methoxyphenols in ethyl acetate, amended onto Teflon filter membranes loaded with $PM_{2.5}$ (see Table 2 for list of deuterated compounds). The solvent was allowed to evaporate for 1 h, and then the filter was extracted with 30 mL methylene chloride/acetone (1:1) in an ultrasonic bath. The extracts were analyzed by GC/MS−SIM after reducing the extract volume to 0.5 mL. Recoveries of all compounds except syringaldehyde were low (median 7%, range 2−45%) because of evaporative losses. Therefore, in all subsequent experiments, the recovery standards were amended onto the filters in glass headspace vials, which were immediately sealed.

The ability of several solvents and solvent mixtures to extract methoxyphenols from clean Teflon filter membranes was evaluated. Deuterium-labeled methoxyphenols in ethyl acetate were amended onto clean Teflon filter membranes and the solvent was allowed to evaporate in glass headspace vials for 1 h, before extraction and analysis. Good recoveries for all compounds were obtained when ethyl acetate was used as the solvent, (median 97%, range 92−111%) but recoveries for the alkyl-substituted syringols were poor when acetone (median 13%, range 8−15%) or methylene chloride: acetone (1:1) (median 31%, range 23−33%) was used as the solvent.

On the basis of the above observations, ethyl acetate was selected as a solvent of choice for extraction of methoxyphenols from PM. Ten outdoor $PM_{2.5}$ samples were analyzed for methoxyphenols. Deuterium-labeled compounds were amended onto the filters prior to analysis. Recoveries of the α-carbonyl substituted methoxyphenols were greater than 100% (median 202%, range 118−302%). The high recovery values may be due to formation of these compounds from the breakdown of other methoxyphenols, as the recoveries of most of the other methoxyphenols were low and variable (median 14%, range 0−162%). In an attempt to identify causes of the variable recoveries, we further analyzed the extracts with the mass spectrometer operated in scan mode. We identified substantial amounts of *o*- and *p*-nitrophenol and a putatative NO_2-adduct of the 2-chloro-4-methoxyphenol ISTD in many of these extracts. The levels of both of the NO_2-derivatives detected in the extracts were inversely correlated with the recoveries of the guaiacol-type and syringol-type deuterated methoxyphenols (Figure 1 and Table 3), whereas recoveries of the α-carbonyl-substituted compounds showed a positive association with levels of the nitrophenol derivatives ($r = 0.67$, Table 3). The syringol-type compounds were more readily degraded than the guaiacol-type compounds. These data imply that some PM components are capable of nitrating the methoxyphenols under relatively mild conditions.

We also investigated the recovery of methoxyphenols from PM standard reference materials. Deuterium-labeled methoxyphenols in ethyl acetate were amended onto urban dust PM samples (SRM1649a) and the solvent was allowed to

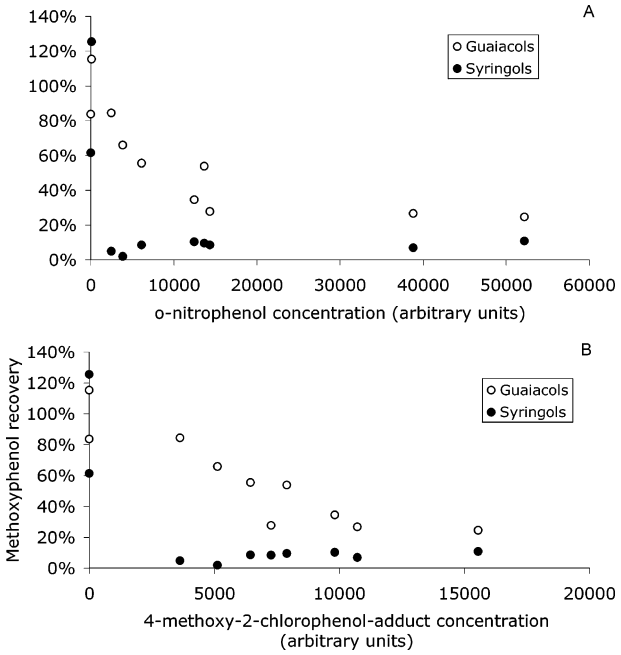

FIGURE 1. Association between methoxyphenol recovery and presence of nitrophenol derivatives in extracts of ambient PM. (A) *o*-nitrophenol; (B) NO_2-adduct of 2-chloro-4-methoxyphenol.

TABLE 3. Correlations between Deuterated Methoxyphenol Recoveries and Presence of Nitrophenol Derivatives in Extracts of Ambient PM Samples

	pearson *r*		
	avg recovery of guaiacols[a]	avg recovery of syringols[b]	avg recovery of α-carbonyls[c]
o-nitrophenol	−0.74[d]	−0.89[e]	0.363
NO_2-adduct of 2-chloro-4-methoxyphenol	−0.37	−0.64[d]	0.674[d]

[a] Sum of d_4-guaiacol, d_3-4-methylguaiacol, d_3-4-ethylguaiacol, d_3-eugenol, and d_3-4-propylguaiacol. [b] Sum of d_3-syringol, d_2-4-methylsyringol, d_2-4-ethylsyringol, and d_2-4-propylsyringol. [c] Sum of d_2-vanillin, d_6-acetovanillone, and d_2-syringaldehyde. [d] $p < 0.05$. [e] $p < 0.01$.

evaporate for 1 h before extraction and analysis. As was observed with the ambient fine PM samples, recoveries of the α-carbonyl-substituted methoxyphenols were somewhat elevated (median 104%, range 103−142%), whereas the recoveries for the other methoxyphenols were poor (guaiacols: median 27%, range 24−72%; syringols: median 3%, range 2−6%). Similar results were obtained using the following solvent mixtures: methylene chloride:acetone (1:1), methylene chloride:ethyl acetate (1:1), methylene chloride:methanol:hexane (1:1:1). In contrast, when deuterated methoxyphenols were amended onto diesel PM samples (SRM2975), recoveries of most of the deuterated compounds from diesel PM were good (median 80%, range 73−130%), except for the alkyl substituted syringols (median 14%, range 11−49%).

Together, these initial studies consistently showed that, in the presence of PM, the methoxyphenols were susceptible to chemical degradation, through nitration and oxidation, during the extraction procedure. The specific components of PM responsible for this degradation were not identified. We were able to reproduce this degradation by treating ethyl acetate solutions containing methoxyphenols with HNO_3 (0.005%) or NO_2 (50 mL, 40 ppm v/v). In these experiments, we observed degradation products consistent with nitration of the aromatic ring, oxidation, and ring opening. Oxidative cleavage of alkenes by nitric acid produces carboxylic acids via a ketol (*29*). Dilute nitric acid readily nitrates methylguaiacol and produces catechols, benzoquinone, and oxalic acid through oxidation (*30*). Products from incomplete oxidation, vanillin and syringaldehyde, were observed and likely came from oxidation of the alkene-substituted guaiacols and syringols: isoeugenol, eugenol, coniferylaldehyde, and sinapylaldehyde. The same products were found after nitric acid oxidation of wood (*31*); lignin in wood contains alkenyl polymers of guaiacol and syringol. Our instrumental analysis conditions would not efficiently detect carboxylic acids (e.g., vanillic acid). The relative reactivity of the alkene substituents can be expected to increase with conjugation and explains the rapid oxidation of α,β-unsaturated aldehydes (e.g., coniferylaldehyde), an observation also noted in wood lignin oxidation by nitric acid (*32*). Also, the rate of degradation was fastest for syringol and its alkyl-substituted analogues, intermediate for guaiacol and alkyl-substituted guaiacols, and slowest for α-carbonyl substituted compounds. This order of reactivity is consistent with the results we observed for the ambient PM samples and can be rationalized by the electronic effects of the ring substituents on ring substitution reactions: methoxy substituents moderately activate arene substitution reactions, the effect being greater when two methoxy groups are present ortho and para to the unsubstituted ring positions, as in the syringols. In contrast, α-carbonyls are strongly deactivating.

On the basis of our understanding of the chemistry described above, we sought to improve the recovery of the methoxyphenols by interfering with the chemical reactions leading to their breakdown. Therefore, we evaluated the ability of several solvent additives to inhibit breakdown of the methoxyphenols. Compounds evaluated included acids, bases, antioxidants, and radical scavengers. Triethylamine (3.6 mM in ethyl acetate) was effective at preventing degradation of the methoxyphenols, and it did not interfere with subsequent GC/MS analysis. Using this modified extraction solvent, good recoveries for deuterium-labeled methoxyphenols amended onto SRM1649a were obtained, (median 95% range 65−96%).

We compared GC/MS analysis of methoxyphenols both as free phenols and as the acetate derivatives. We found that acetylation of the phenols improved chromatographic peak shape, increased sensitivity, and reduced the frequency with which the front end of the GC column and the injector had to be maintained. However, the acetylation reaction was relatively slow for the syringol-type compounds and required heating at 70 °C for ∼3 h for complete reaction. Typical ion chromatograms for our standard mixture and a representative (outdoor) environmental sample are illustrated in Figure 2.

The improved procedure for determination of methoxyphenols in ambient PM was evaluated when applied to samples of PM collected from an urban air monitoring site (Beacon Hill, Seattle, WA). Results are presented in Table 4 along with recovery data and limits of quantitation. The limit of quantitation, defined for each analyte as the greater of the average concentration of analyte detected in blank filter extracts or the analytical limit of detection at $s/n = 2$, was calculated assuming a 14.4 m^3 sample (10 L/min × 24 h). The limits of quantitation for guaiacol, vanillin, and acetovanillone were higher than the other compounds because of the presence of variable levels of these analytes in blank filter extracts. The precision of this assay, on the basis of replicate measurements of the deuterated recovery standards (at an effective concentration of ∼3 ng/m^3) was 2−6%. The data have not been adjusted on the basis of recovery of the deuterated compounds, because deuterated surrogates were not available for all analytes.

Using the modified extraction solvent, acceptable recoveries were obtained for all the recovery standards, in all the samples (median 74%, range 65−100%). Multiple methox-

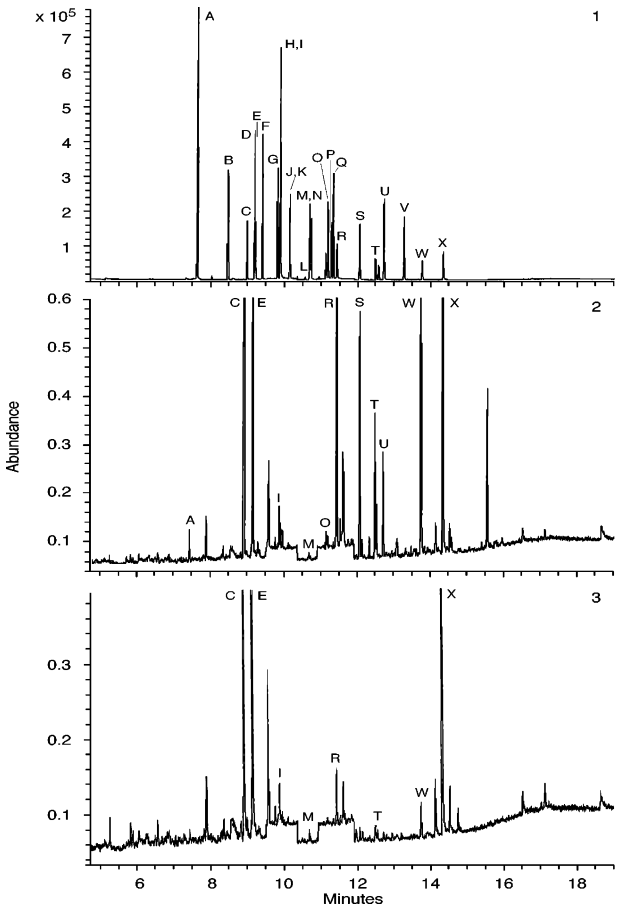

FIGURE 2. GC/MS selected ion chromatograms for (1) methoxyphenol standard mixture, (2) extract of wood smoke source sample from open fire, and (3) extract of ambient $PM_{2.5}$ sample. Compounds are acetates unless otherwise stated. Annotation key: (A) guaiacol, (B) 4-methylguaiacol, (C) 2,3-dimethoxyphenol, (D) 4-ethylguaiacol, (E) 2-chloro-4-methoxyphenol, (F) syringol, (G) eugenol, (H) 4-propylguaiacol, (I) vanillin, (J) *cis*-isoeugenol, (K) 4-methylsyringol, (L) *trans*-isoeugenol, (M) acetovanillone, (N) 4-ethylsyringol, (O) guaiacylacetone, (P) 4-allylsyringol, (Q) 4-propylsyringol, (R) syringaldehyde, (S) 4-acetosyringone, (T) coniferyladehyde, (U) 4-propylsyringone, (V) 4-butylsyringone, (W) sinapylaldehyde, and (X) sinapylaldehyde propionate.

yphenols were detected in the low ng/m^3 range with vanillin, syringaldehyde, coniferylaldehyde, and sinapylaldehyde being the most abundant compounds. Comparison of the current data with previously reported measurements of methoxyphenols in ambient samples is difficult because differences in sample collection methods may influence the comparability. Our observed methoxyphenol levels are lower than 1988/1989 wintertime ambient levels in Minneapolis (e.g., syringaldehyde: median 65 ng/m^3, range 6.1−142 ng/m^3) and Salt Lake City (syringaldehyde: median 6.8 ng/m^3, range 0.1−23 ng/m^3) reported by Hawthorne et al. (*17*), although Hawthorne's data include both particle-bound and vapor-phase chemical (our measurements only capture particle-bound methoxyphenols). Our reported methoxyphenol levels are also lower than wintertime (1995) levels of particle-bound methoxyphenols reported by Schauer et al. at urban sites (Fresno and Bakersfield) in the San Joaquin Valley (e.g., 11−135 ng/m^3 for syringaldehyde) but are similar to levels reported for a rural site in the Kern Wildlife Refuge (syringaldehyde 0.3 ng/m^3) (*3*). Combustion of wood for residential heating is common in Minneapolis, Salt Lake City, and Fresno (*2*, *17*). While wood burning is also a major source of PM in Seattle, peak wintertime excursions in PM have been greatly reduced in recent years as a consequence of

TABLE 4. Analytical Performance and Methoxyphenol Concentrations in Ambient $PM_{2.5}$ (n = 10) from Seattle, WA

compd	recovery of deuterated standards (% ± 1 SD)	limit of quantification (ng/m^3)	median and range (ng/ m^3)
guaiacol	65 ± 2	0.45	0.64 (0.55−0.67)
methylguaiacol	79 ± 4	0.06	0.06 (<0.06−0.07)
ethylguaiacol	70 ± 2	0.07	0.13 (<0.07−0.15)
syringol	70 ± 3	0.08	0.13 (<0.08−0.14)
eugenol	73 ± 4	0.13	0.34 (0.32−0.43)
propylguaiacol	75 ± 3	0.09	<0.09
vanillin	63 ± 2	0.25	0.96 (0.52−1.45)
methylsyringol	83 ± 5	0.07	0.23 (<0.07−0.35)
acetovanillone	79 ± 4	0.17	0.22 (<0.17−0.25)
ethylsyringol	100 ± 6	0.03	0.27 (<0.13−0.32)
allylsyringol		0.08	<0.08
propylsyringol	89 ± 4	0.08	0.19 (<0.08−0.23)
syringaldehyde	87 ± 5	0.08	0.61 (0.08−1.52)
acetosyringone		0.07	0.47 (<0.07−1.06)
coniferylaldehyde		0.10	0.42 (<0.10−1.16)
4-propylsyringone		0.07	0.31 (<0.07−0.54)
4-butylsyringone		0.07	<0.07
sinapylaldehyde		0.12	0.56 (<0.12−1.43)

regulations which restrict residential wood burning when unfavorable atmospheric conditions exist. (*1*). The median $PM_{2.5}$ concentration for the ambient PM samples in Table 4 was 18 μg/m^3 (range 7−23 μg/m^3); Schauer's samples were collected in part during a relatively severe air pollution episode in 1995 in Fresno, CA ($PM_{2.5}$ levels up to 55 μg/m^3) (*3*). The methoxyphenols more volatile than syringaldehyde exist predominantly in the vapor phase at ambient temperatures (*3*). Hence, the concentrations of lower molecular weight methoxyphenols (e.g., guaiacol, syringol) observed in the current study only account for the amount bound to particles and retained on the filter. Our efforts to capture these compounds on polyurethane foam (PUF) at 10 LPM over 24 h, adapted from Hawthorne et al. (*14*, *17*), were not successful. Breakthrough of guaiacol and syringol with the PUF sorbent occurred at very low sample volumes at ambient temperature (<15 m^3 for a 30-mL PUF cartridge when sampled for 24 h at 10 L/min.). We are continuing research toward identifying a sorbent with appropriate higher affinity for the vapor-phase methoxyphenols.

Using the modified extraction solvent, nitration or oxidation of the methoxyphenols was not observed during sample extraction and analysis. Although nitration of other aromatic compounds in the atmosphere is well established (*33*), we are not aware of reports of nitrated methoxyphenols in ambient PM samples. Clearly, the ease with which several of the methoxyphenols were degraded under relatively mild conditions in the laboratory has important implications for the use of those compounds as tracers for wood smoke in environmental PM samples. Future research is required to identify the PM components responsible for the degradation of the methoxyphenols and to determine whether the degradation takes place in the air prior to sample collection, on the filter media during sample collection or subsequent storage, or only during sample extraction and analysis. Hawthorne et al. reported that methoxyphenols bearing alkenyl substituents (e.g., isoeugenol, allylsyringol) were depleted in ambient samples relative to source samples collected from residential chimneys, whereas the α-carbonyl-substituted compounds vanillin and syringaldehyde were proportionally enriched in the ambient samples (*17*). Thus, Hawthorne's data suggest that specific methoxyphenols, in particular those bearing alkenyl substituents, chemically degrade in (outdoor) air. Until quantitative measures of the stability of methoxyphenols, both in the air and adsorbed on

TABLE 5. Concentrations of Collocated $PM_{2.5}$ and PM_{10} (in $\mu g/m^3$) and Methoxyphenols and Levoglucosan (in ng/m^3)

sample location	sample type	PM mass	levoglucosan	vanillin	syringaldehyde	acetosyringone	sinapylaldehyde
outdoor	PM_{10}	32	39	0.24	0.11	0.02	1.47
	$PM_{2.5}$	26	51	0.25	0.10	nd[b]	1.30
outdoor	PM_{10}	40	35	0.29	0.27	0.05	1.61
	$PM_{2.5}$	25	37	0.29	0.22	0.02	1.56
outdoor	PM_{10}	35	13	0.18	0.09	nd	0.88
	$PM_{2.5}$	26	15	0.22	0.13	0.02	0.75
indoor	PM_{10}	36	33	0.26	0.19	0.04	1.65
	$PM_{2.5}$	32	26	0.22	0.17	0.04	1.89
indoor	PM_{10}	39	63	0.40	0.48	0.11	1.22
	$PM_{2.5}$	26	63	0.48	0.45	0.15	1.27
indoor	PM_{10}	38	15	0.19	0.19	nd	0.97
	$PM_{2.5}$	27	20	0.29	0.25	nd	0.83
indoor	PM_{10}	37	28	0.33	0.24	0.02	1.09
	$PM_{2.5}$	25	18	0.25	0.19	nd	0.77
indoor	PM_{10}	29	762	1.60	1.66	1.05	5.13
	$PM_{2.5}$	24	700	1.45	1.47	0.88	4.62
indoor	PM_{10}	30	141	0.96	0.58	0.17	1.44
	$PM_{2.5}$	23	138	0.79	0.48	0.11	0.73
mean	PM_{10}	35	125	0.49	0.42	0.21	1.72
	$PM_{2.5}$	26	119	0.47	0.38	0.20	1.52
p-value[a]		<0.0001	0.77	0.48	0.16	0.30	0.08

[a] Paired t-test. [b] nd, not detected.

filter media, are established, reported ambient concentrations of methoxyphenols should be interpreted with caution.

Various filter media, most commonly Teflon and quartz, are used to collect samples of atmospheric PM. To determine the general applicability of this assay, we determined recoveries of deuterated methoxyphenols (~40 ng) amended onto clean Teflon and quartz filters ($n = 5$). Median methoxyphenol recoveries from Teflon were 92% (range 83−132%). Recoveries of most of the deuterated methoxyphenols from quartz filters were also acceptable (median 98%, range 87−131%), except for the syringol-type compounds (median 46%, range 33−72%). During development of the method, we observed that syringol and its alkyl-substituted analogues are more susceptible to chemical transformation via ring-substitution reactions than other methoxyphenols. These data indicate that Teflon membranes should be the preferred sample collection media for analysis of organic tracers in PM samples. The use of quartz filters to collect PM samples may compromise subsequent quantitative analysis for specific methoxyphenols and potentially for other reactive organic chemicals.

Determination of Methoxyphenols in PM. The newly developed and validated analytical procedure for determination of methoxyphenols in PM was applied to ambient PM samples collected in Seattle, WA. As part of the exposure panel study, co-located $PM_{2.5}$ and PM_{10} samples were collected and $PM_{2.5}$ samples were subject to trace element analysis with XRF (*24*). This raises two issues when multiple analyses are needed from the same filter samples: Do the $PM_{2.5}$ samples give similar concentrations of methoxyphenols before and after the XRF? If not, do the co-located PM_{10} and $PM_{2.5}$ samples give similar methoxyphenols measurements such that the co-located PM_{10} samples not subject to XRF could be used for analysis of methoxyphenols? We hypothesized that $PM_{2.5}$ and PM_{10} samples give similar methoxyphenol levels because the vast majority of wood smoke particle mass is associated with particles with aerodynamic diameters $<1\ \mu m$ (PM_1) (*34*). Nine pairs of co-located $PM_{2.5}$ and PM_{10} samples that had not been subjected to XRF analysis were analyzed for methoxyphenols. The results are summarized in Table 5. The anhydro-sugar levoglucosan, a product of pyrolysis of cellulose and a putatative marker for wood smoke PM, has previously been reported in this same set of samples (*28*), and the data are included in Table 5 for comparison with the methoxyphenols.

Only the relatively nonvolatile carbonyl-substituted methoxyphenols were reliably detected in these environmental samples. Syringol and guaiacol, and their methyl, ethyl ethenyl, and propyl substituted analogues exist to a large extent in the gas phase and were rarely present above the quantification limit in PM samples. There is no significant difference between methoxyphenol concentrations (expressed as ng/m^3) as determined from co-located PM_{10} and $PM_{2.5}$ samplers using either a paired t-test or a nonparametric sign test.

On the basis of the above findings, we analyzed 10 pairs of co-located ambient $PM_{2.5}$ and PM_{10} samples in which one sample from each pair had already undergone XRF analysis.

TABLE 6. Effect of XRF Analysis on Subsequent Determination of Methoxyphenol Concentrations in Co-Located Ambient PM[a]

site no.	XRF treatment	vanillin	acetovanillone	syringaldehyde	acetosyringone	coniferylaldehyde	sinapylaldehyde
1	+	0.99	0.52	1.14	0.57	1.63	1.04
	−	1.10	0.49	1.41	0.72	1.81	0.99
2	+	1.62	0.49	1.62	0.62	1.75	2.39
	−	1.81	0.50	1.96	0.74	1.86	2.63
3	+	3.21	0.85	6.67	3.92	5.01	12.23
	−	3.66	0.76	8.64	5.05	6.13	14.97
4	+	2.60	0.63	6.31	3.96	5.01	12.99
	−	3.13	0.77	8.51	5.13	5.99	15.54
5	+	2.73	0.82	5.62	3.07	5.08	15.75
	−	3.33	0.77	7.48	4.48	6.82	21.87
6	+	3.07	0.66	6.69	3.96	5.96	15.62
	−	3.74	1.69	9.45	6.69	9.49	22.29
7	+	2.54	0.63	7.28	5.05	5.22	12.90
	−	2.38	0.66	6.93	4.97	5.05	11.97
8	+	1.08	0.55	1.17	0.52	1.64	3.74
	−	1.35	0.51	1.56	0.69	1.81	4.29
9	+	0.68	0.45	0.70	0.32	nd	1.10
	−	0.77	0.40	0.77	0.35	nd	1.22
10	+	1.46	0.52	1.14	0.52	1.80	2.01
	−	1.50	0.51	1.21	0.55	1.84	1.89
mean	+	2.00	0.61	3.83	2.25	3.68	7.98
	−	2.28	0.71	4.79	2.94	4.53	9.77
p-value		0.01	0.39	0.02	0.04	0.06	0.07

[a] Concentrations in ng/m^3.

Although XRF analysis is considered "nondestructive", we were concerned that the XRF analysis may cause degradation or volatilization of organic chemical species on the filter, thus compromising subsequent analysis of methoxyphenols in the PM samples. XRF analysis was performed per batch of 12 filters placed in the XRF spectrometer under vacuum (0.15 Torr) for 12 h. During the 12-h period, each filter was subjected to X-ray bombardment for a total of 45 min, with beam energies of 7.5−55 kV. Filters were stored at −4 °C prior to and immediately after the XRF analysis. The results are summarized in Table 6.

The co-located samples were either both $PM_{2.5}$ samples (sites 1−3) or co-located PM_{10} and $PM_{2.5}$ samples (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. On the basis of our conclusions from Table 5, co-located PM_{10} and $PM_{2.5}$ samples were treated as equivalent samples for the paired t-test. As shown in Table 6, concentrations of vanillin, syringaldehyde, and acetosyringone were significantly lower in the filter subjected to XRF analysis when compared to the corresponding co-located sample that was not subjected to XRF analysis. Levels of coniferylaldehyde and sinapylaldehyde were also decreased in the XRF treated samples, and this difference approached statistical significance. Similar results were obtained when nonparametric methods (sign test) were used to analyze the data in Table 6. These observations are in contrast to our recent report that XRF analysis of ambient PM samples did not affect subsequent determination of levoglucosan (*28*). It is possible that the energy of the X-ray beam causes chemical transformation of the methoxyphenols. Alternatively, the reduction in methoxyphenol levels we observed following XRF treatment may be due to evaporation of the semivolatile methoxyphenols either from direct heating by the X-ray beam or during the 12 h the filters are under vacuum inside the XRF spectrometer. The vacuum inside the XRF spectrometer is 0.15 Torr. In comparison, the pressure drop across the HI_{10} and $HI_{2.5}$ used to collect the samples in the present study was 27 and 29 Torr, respectively, and the pressure drop across a high-volume sampler (e.g., Chem-vol) may be as high as 100 Torr. Although the pressure drop across typical PM samplers does not approach the vacuum inside the XRF spectrometer, it is nevertheless probable that the accuracy of measurements of particle-bound methoxyphenols that are collected by typical ambient PM samplers may be compromised by a significant desorption artifact.

In conclusion, we developed an improved analytical method that allows for quantitative determination of methoxyphenols in samples of ambient PM, with good recoveries and low limits of detection. We observed that ambient PM and PM standard reference materials could cause oxidation and nitration of methoxyphenols during sample extraction; however, this degradation could be prevented by using a solvent additive (triethylamine). The use of deuterium-labeled authentic standard compounds to monitor methoxyphenol recovery is a critical feature of this method; our recovery data demonstrate acceptable reproducibility and precision for determination of methoxyphenols in environmental samples collected on PTFE membrane filters. We also demonstrated that methoxyphenols are present predominantly in the fine ($PM_{2.5}$) particle size fraction and that XRF analysis of samples compromised subsequent measurement of methoxyphenol levels. Our study underscores the fact that many methoxyphenols are chemically reactive and exhibit appreciable volatility—two features that could limit the utility of these chemicals in source attribution studies. Future studies are called for to measure the atmospheric stability of the methoxyphenols and to assess the influence of adsorption and desorption artifacts on measurements of methoxyphenols.

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Literature Cited

(1) Maykut, N. N.; Lewtas, J.; Kim, E.; Larson, T. V. *Environ. Sci. Technol.* **2003**, *37*, 5135−5142.
(2) Poore, M. W. *J. Air Waste Manage. Assoc.* **2002**, *52*, 3−4.
(3) Schauer, J. J.; Cass, G. R. *Environ. Sci. Technol.* **2000**, *34*, 1821−1832.
(4) Brauer, M.; Hisham-Hashim, J. *Environ. Sci. Technol.* **1998**, *32*, 404A−407A.
(5) Mott, J. A.; Meyer, P.; Mannino, D.; Redd, S. C.; Smith, E. M.; Gotway-Crawford, C.; Chase, E. *West J. Med.* **2002**. *176*, 157−162.
(6) Osterman, K.; Brauer, M. In *Forest Fires and Regional Haze in Southeast Asia*; Eaton, P., Radojevic, M., Eds.; Nova Science Publishers: Hauppauge, NY, 2001; Chapter 10.
(7) Smith, T.; Ward, G. Air sampling of the 2000 Montana wildfire season. Presented at the Air and Waste Management Association Annual Conference, 2001; Paper 1131.
(8) Ezzati, M.; Kammen, D. M. *Environ. Health Perspect.* **2001**, *109*, 481−488.
(9) Smith, K. R. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 13286−13293.
(10) Leonard, S. S.; Wang, S.; Shi, X.; Jordan, B. S.; Castranova, V.; Dubick, M. A. *Toxicology* **2000**, *150*, 147−157.
(11) Pryor, W. A. *Free Radical Biol. Med.* **1992**, *13*, 659−676.
(12) Schauer, J. J.; Kleeman, M. J.; Cass, G. R.; Simoneit, B. R. *Environ. Sci. Technol.* **2001**, *35*, 1716−1728.
(13) Larson, T. V.; Koenig, J. Q. *Annu. Rev. Public Health* **1994**, *15*, 133−156.
(14) Hawthorne, S. B.; Krieger, M. S.; Miller, D. J.; Mathiason, M. B. *Environ. Sci. Technol.* **1989**, *23*, 470−475.
(15) Rogge, W. F.; Hildemann, L. M.; Mazurek, M.; Cass, G. R.; Simoneit, B. R. T. *Environ. Sci. Technol.* **1998**, *32*, 13−22.
(16) Fine, P. M.; Cass, G. R.; Simoneit, B. R. *Environ. Sci. Technol.* **2002**, *36*, 1442−1451.
(17) Hawthorne, S. B.; Miller, D. J.; Langenfeld, J. J.; Krieger, M. S. *Environ. Sci. Technol.* **1992**, *26*, 2251−2262.
(18) Nolte, C. G.; Schauer, J. J.; Cass, G. R.; Simoneit, B. R. *Environ. Sci. Technol.* **2001**, *35*, 1912−1919.
(19) Kjällstrand, J.; Ramnas, O.; Petersson, G. *J. Chromatogr., A* **1998**, *824*, 205−210.
(20) Dills, R. L.; Zhu, X.; Kalman, D. A. *Environ. Res.* **2001**, *85*, 145−158.
(21) Coscia, C. J.; Schubert, W. J.; Nord, F. F. *J. Org. Chem.* **1961**, *26*, 5085−5091.
(22) Buu-Hoï, N. P.; Séalles, J., Jr. *J. Org. Chem.* **1955**, *20*, 606−609.
(23) Chau, A. S. Y.; Coburn, J. A. *J. Assoc. Off. Anal. Chem.* **1974**, *57*, 389−393.
(24) Liu, L. J.; Box, M.; Kalman, D.; Kaufman, J.; Koenig, J.; Larson, T.; Lumley, T.; Sheppard, L.; Wallace, L. *Environ. Health Perspect.* **2003**, *111*, 909−918.
(25) Marple, V. A.; Rubow, K. L.; Turner, W.; Spengler, J. D. *J. Air Pollut. Control Assoc.* **1987**, *37*, 1303−1307.
(26) Allen, R.; Box, M.; Liu, L.-J. S.; Larson, T. *J. Air Waste Manage. Assoc.* **2001**, *51*, 1650−1653.
(27) Fenimore, D. C.; Davis, C. M.; Whitford, J. H.; Harrington, C. A. *Anal. Chem.* **1976**, *48*, 2289−2290.
(28) Simpson, C. D.; Dills, R. L.; Katz, B. S.; Kalman, D. A. *J. Air Waste Manage. Assoc.* **2004**, *54*, 689−694.
(29) Franz, J. E.; Herber, J. F.; Knowles, W. S. *J. Org. Chem.* **1965**, *30*, 1488−1491.
(30) Sobolev, I. *J. Org. Chem.* **1961**, *26*, 5080−5085.
(31) Brink, D. L.; Bicho, J. G.; Merriman, M. M. *Adv. Chem. Ser.* **1966**, *59*, 177−204.
(32) Polcin, J.; Rapson, W. H. *Pulp Pap. Can.* **1971**, *72*, T114−T125.
(33) Atkinson, R.; Arey, J. *Environ. Health Perspect.* **1994**, *102* (Suppl 4), 117−126.
(34) Kleeman, M. J.; Schauer, J. J.; Cass, G. R. *Environ. Sci. Technol.* **1999**, *33*, 3516−3523.

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