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Fractionation of polar organic extracts of airborne particulate matter using cyanopropyl-bonded silica in solid-phase extraction

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ABSTRACT

A mg-scale fractionation method has been developed for polar organic matter in airborne particles. The method gives reproducibly good recoveries of mass while avoiding the use of water or salts. Cyanopropyl-bonded silica solid-phase extraction (SPE) columns were used to fractionate a mixture of standard compounds and acetone-soluble extracts from particles collected in Elizabeth, NJ, USA and from National Institute of Standards and Technology Standard Reference Material SRM 1649 (urban air particles). Critical factors proved to be reducing the polarity of the extract before its application to the column and pre-wetting the column with *n*-hexane. Ten fractions were eluted with solvent mixtures of increasing polarity, ranging from *n*-hexane to methanol. Blank-corrected mass recoveries were 95 and 98% for the Elizabeth, NJ, USA and SRM 1649 extracts, respectively.

INTRODUCTION

In this paper the term particulate polar matter (PPOM) refers to acetone-soluble material extracted from samples of ambient airborne particles after they have already been extracted with the less polar solvent dichloromethane (or cyclohexane followed by dichloromethane) [1,2]. PPOM accounts for 30 to 60% of the organic-solvent extractable mass of airborne particles [1,2] and 30 to 50% of the direct-acting mutagenic activity in the Ames bioassay with TA-98 [3,4]. However, relatively little work has been done to date to chemically characterize this material or to develop fractionation methods for it. This is because of the difficulties in working with complex mixtures of polar organic compounds.

We chose acetone extracts because some characterization and bioassay data were available for the (unfractionated) acetone extracts used in this study. Methanol extracts have also been characterized to some extent [5,6]. Acetone has been found to extract less inorganic and more organic material from Standard Reference Material SRM 1649, urban air particles, than methanol [5,7]. Acetone extracts of SRM 1649 also had higher mutagenic activity (in revertants per gram of extract) than methanol extracts [5,7].

The goal of the research reported here was to develop a mg-scale fractionation method for particulate polar organics based on polarity, with reproducibly good mass recovery, while avoiding addition of water or salts to the fractions. Evaporation of water or other high-boiling solvents to concentrate samples for bioassay and chemical characterization is very difficult, as is salt removal. Neither normal-phase chromatographic separation on silica or alumina nor reversed-phase separation on C_{18} columns could meet these requirements. Substantial losses of mass could be expected from use of a normal-phase silica or alumina column. Losses of mutagenic activity have also been reported for these sorbents [8,9]. Such losses would not be expected

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for a C_{18} reversed-phase system; however, such systems typically use water as one of the solvents for the separation. Reversed-phase columns also require the presence of buffer salts to control the ionization of polar compounds and thus permit reproducible retention behavior of polar compounds.

Normal-phase separation on cyano-bonded silica was investigated, based on the nature of the organic materials and the need for elution solvents that could be easily evaporated, *e.g.*, *n*-hexane, dichloromethane or methanol [10]. Lafleur and co-workers [9] have reported good recoveries of mass and mutagenic activity for dichloromethane extracts of combustion aerosols which were separated on cyanopropyl solid-phase extraction (SPE) columns. Lafleur and Nakagawa [11] also found good mass recovery when they used cyanopropyl SPE columns to fractionate pyridine extracts of bituminous coal. This is the first study to use cyano-bonded silica for more polar organic materials found in the acetone-soluble fraction of ambient aerosols.

SPE offered the possibility of fractionation of mg quantities of material in a single application. This is in contrast to more time- and solvent-consuming fractionation methods based on fraction collection from cyano-bonded columns in high-performance liquid chromatography [12,13].

EXPERIMENTAL

Sources of particles, sampling, extraction, storage of extracts

Two types of particulate matter were used in this investigation: filter samples of inhalable particulate matter collected in Elizabeth, NJ, USA [14] during the winter of 1983 and National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1649, Washington Urban Air Particulate Material. The Elizabeth samples were collected on pre-cleaned glass fiber filters for 24-h periods over a 6-week period, using a hi-volume air sampler with a size-selective inlet (D_{50} = 15 μm). Individual filters were Soxhlet-extracted sequentially with cyclohexane, dichloromethane, and acetone [1,2,14]. Each extract was filtered and reduced in volume to 10.0 ml using a rotary evaporator. Extract masses were determined with a Cahn microbalance, Model 25, by weighing the residue of duplicate 100-μl aliquots of the extracts taken to dryness on a slide warmer at 45°C. Samples were stored in a freezer at −30°C, in the dark. For this research, a composite sample of the acetone-soluble extracts from Elizabeth, NJ, USA, collected during winter 1983, was prepared based on equal air volumes for each of the 39 days of the sampling period. The total composite mass was 479 mg, corresponding to sampling 44 400 m^3 air over a 6-week period. The SRM 1649 acetone extract was prepared by sequential Soxhlet extraction of a 1-g portion of particles with dichloromethane followed by acetone, filtration and rotary evaporation of excess solvent. Extract samples were stored in the freezer. Previous work had shown that the mass of material extracted with dichloromethane is equivalent to the sum of the masses extracted with cyclohexane followed by dichloromethane [15].

Fractionation on cyanopropyl-bonded phase columns

SPE solid-phase extraction columns containing 500 mg of cyanopropyl-bonded silica packing (J.T. Baker) were used. They were pre-cleaned with 10 ml of chromatographic-grade solvents, in order: *n*-hexane, ethyl acetate, dichloromethane, and methanol. The columns were covered loosely with aluminum foil and dried overnight. Prior to sample application, the columns were wetted with 2 ml of *n*-hexane. The acetone extract of the Elizabeth sample was evaporated to near dryness, redissolved in methanol and then evaporated to near dryness again. This was done to remove acetone which has appreciable ultraviolet absorbance and interferes with characterization by high-performance liquid chromatography. Methanol, dichloromethane and *n*-hexane were added to the dried extract, in that order, so that the final solvent proportions were 1:1.2:1.4. This mixture of solvents dissolved the extract completely and kept the extract in a narrow band at the top of the column.

Before application to the column the mass density of the mixture was determined for duplicate dried 100-μl aliquots, using a Cahn microbalance. An amount of 4 mg of extract in 200 μl of solvent mixture was applied to the top of a pair of coupled columns which had been wetted with *n*-hexane. The loaded columns dried in air overnight. A series of elution solvent mixtures of increasing polarity was then passed through the coupled columns, and the eluent fractions were collected and evaporated to

dryness. Methanol (500 μl) was added to each fraction and the dry mass of two 100-μl portions was determined. The elution solvents were: *n*-hexane, 25% dichloromethane in *n*-hexane (2 aliquots), 100% dichloromethane (2 aliquots), 2%, 5%, 15% and 40% methanol in dichloromethane, and 100% methanol. The aliquots were each 2 ml, except for the methanol which was 3 or 6 ml. Air pressure was applied to the top of the coupled columns to keep the solvent flow-rate at about 2 ml/min.

The fractionation and mass determination were done with three separate aliquots of the Elizabeth winter 1983 extract. The fractions were labelled A–J in order of increasing elution solvent polarity. Column blanks were eluted the same way and the mass in each recovered fraction determined for a blank correction. The fractions were characterized by HPLC with ultraviolet (UV) detection. A 4-mg sample of the acetone extract of NBS 1649 particles was also fractionated and characterized as described above. The capacity of 500-mg SPE columns was determined by adding up to 6.2 mg of the Elizabeth extract mixture and assessing the quality of the separation visually.

The fractionation procedure was performed on a mixture of these standard compounds (obtained from Aldrich): 5-nitrovanillin, vanillin, 2-naphthoic acid, 1,4-dihydroxynaphthalene, 5-nitroquinoline, 2-nitro-6H-dibenzo[*b*,*d*]pyran-6-one (6-nitro-3,4-benzocoumarin), 2-nitronaphthalene, 5,6-benzoquinoline, acridine, pyrene carboxaldehyde and dioctylphthalate. The standards were prepared in either methanol or acetonitrile, but the mixture was evaporated to dryness and reconstiuted in 0.45 ml of *n*-hexane, dichloromethane and methanol in the proportions given above, before application to a single clean, *n*-hexane-wetted cyano SPE column. Elution and analysis procedures were the same as for the extracts of ambient particles. Two aliquots of the standard mixture were fractionated. In one experiment, evaporation losses were evaluated by determining recovery of the standard compounds after reconstitution and before fractionation. In another experiment the Elizabeth acetone extract was spiked with the standard mixture and fractionated.

High-performance liquid chromatography

Standard compounds, whole extracts and fractions were analyzed on a Hewlett-Packard Model 1090M high-pressure liquid chromatograph equipped with a DR 5 solvent delivery system, a diode array UV–Vis detector, a fluorescence detector (Model 1046A) and Model 79994A LC Workstation software. Samples were chromatographed on a Vydac 201TP52 C_{18} reversed-phase microbore column, 15 cm × 2.1 mm I.D. and 5 μm diameter particles, using gradient elution. The analytical column was preceded by a guard cartridge column filled with 10 μm diameter Vydac 201TP packing material (purchased from Alltech). A 5-μl injection loop was used. The initial solvent composition was 5% acetonitrile in water. From 3 to 13 min the solvent composition was changed linearly to 100% acetonitrile and held there for 5 min. Gradient reversal and column equilibration were complete 25 min after injection. Flow through the column was 0.3 ml/min. Three absorbance wavelengths were used for detection: 205, 230 and 254 nm. The fluorescence detector used an excitation wavelength of 230 nm, and all emitted light above 305 nm was collected.

Retention times were determined for the polar aromatic standard compounds at about 0.4 mg ml^{-1} each in a methanol–acetonitrile mixture (1:5, v/v): 5-nitrovanillin, 1.6 min; vanillin, 4.2 min; 2-naphthoic acid, 6.5 min; 1,4-dihydroxynaphthalene, 7.6 min; 5-nitroquinoline, 8.4 min; 2-nitro-6H-dibenzo[*b*,*d*]pyran-6-one, 12.4 min; 2-nitronaphthalene, 12.8 min; 5,6-benzoquinoline, 13.6 min; acridine, 14.2 min; pyrene carboxaldehyde, 14.6 min; and dioctylphthalate, 16.8 min. Retention times for 5-nitrovanillin, vanillin, naphthoic acid and dihydoxynaphthalene depended on the condition of both the analytical and guard columns and use of freshly filtered and degassed elution solvents. The gradient elution program was modified as necessary to ensure reproducible retention times of standard compounds before the extracts of ambient particles or their fractions were analyzed.

RESULTS

In developing this fractionation method, the behavior of the colored Elizabeth extract on the cyano SPE columns was monitored using various proportions of hexane, dichloromethane and methanol as elution solvents. Initially, visual monitoring of the movement of colored bands on the column and the

mass distribution in the resulting fractions were used to assess the success of the fractionation. Finally, characterization by HPLC was used to assess polarity differences among UV-absorbing components of the fractions. The choice of elution solvents was based on low boiling points, relative chemical inertness and UV absorbance. All fractions were redissolved in methanol because of its UV transparency and compatibility with reversed-phase HPLC. Development of the fractionation method started with actual acetone extracts of ambient particles rather than with standard compounds because very little data is available about the chemical composition of such polar extracts of ambient air particles.

At first we intended to separate the extract mass into four fractions, if possible. We found that ten fractions of increasing polarity led to three separate mass recovery maxima, centered on fractions B, D and H (Table I). Table I shows that B was colorless, but both D and H were yellow. Each group of fractions was separated by at least one fraction which had much lower mass. Such a mass distribution showed promise for future bioassay and chemical characterization studies.

Once the desired fractionation mass distribution had been achieved, three pairs of coupled (500 mg) cyano-bonded silica packed columns were used to fractionate three aliquots (4 mg each) of the acetone extract of the Elizabeth winter particle samples. Critical factors in successful fractionation proved to be reduction of the polarity of the extract before its application to the column and wetting the column with *n*-hexane before application of the extract. The blank-corrected mass distribution is displayed in Table I for the three aliquots of Elizabeth Winter 1983 particles. The total mass recovery, with blank correction, averaged 96 ± 5%. The most polar fractions G, H, I and J, accounted for 61% of the recovered mass. The observed differences in mass distribution among the replicates reflect differences in elution flow-rate, one done at 2 ml min^{-1} and two at about 4 ml min^{-1}. For the latter two, 8 and 6 ml methanol were used, respectively, for the final fraction J, whereas the first used only 3 ml methanol. Larger methanol volumes yielded only a few percent better recoveries.

Each fraction from the first of the three fractionations was characterized by HPLC with diode array detection at 205, 230 and 254 nm. The chromatograms for 205 nm are shown in Fig. 1. Absorbance intensity decreased in the order 205 > 230 > 254 nm for all peaks. The unfractionated extract had two peaks at widely separated retention times, indicating the presence of two strongly-absorbing polarity groups within the acetone extract. The second fraction, B, showed a strong absorbance at the same retention time and wavelength as observed for the polar fraction in the preliminary fractionation by solvent polarity [7]. The fourth fraction, D, had no appreciable absorbance although it contained 10%

TABLE I

FRACTIONATION OF THE ACETONE EXTRACT OF AMBIENT PARTICLES FROM ELIZABETH, NJ, USA

Fraction	Solvent	Color	Intensity[a]	Mass recovery, %[b]	
				Average	S.D.
A	Hexane	Clear	0	1.2	0.1
B	25% $MeCl_2$	Clear	0	8.6	0.4
C	25% $MeCl_2$	Faint	0.5	2.7	1.7
D	$MeCl_2$	Light yellow	3	9.8	1.5
E	$MeCl_2$	Light yellow	2	5.8	0.4
F	2% MeOH	Pale	1	4.7	1.2
G	5% MeOH	Yellow	3	16.2	4.0
H	15% MeOH	Yellow	5	31.4	7.1
I	40% MeOH	Light yellow	1.5	13.1	1.2
J	MeOH	Clear	0	6.7	2.6
Total recovery				96	5

[a] Visual estimate; a value of 5 was arbitrarily assigned to the fraction with the most intense color.
[b] Blank-corrected recovery, %.

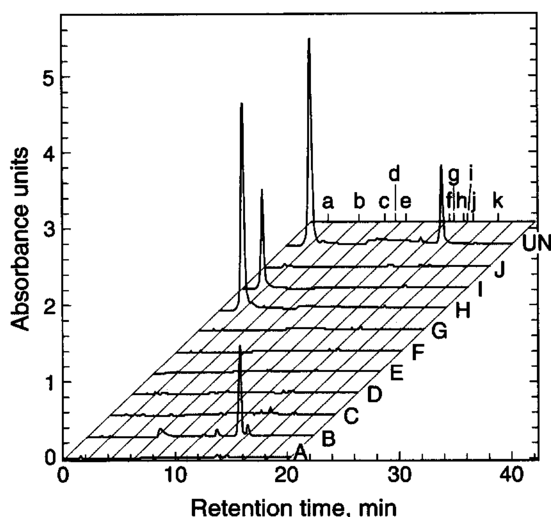

Fig. 1. Reversed-phase chromatograms of the acetone extract of Elizabeth ambient particles. The ordinate shows absorbance at 205 nm in absorbance units. The top chromatogram indicates the retention times for the standard compounds: a = 5-nitrovanillin; b = vanillin; c = 2-naphthoic acid; d = 1,4-dihydroxynaphthalene; e = 5-nitroquinoline; f = 2-nitro-6H-dibenzo[*b*,*d*]pyran-6-one; g = 2-nitronaphthalene; h = 5,6-benzoquinoline; i = acridine; j = 1-pyrenecarboxaldehyde, and k = dioctylphthalate. The unfractionated extract is labelled as UN; fractions are identified by capital letters at the right of each chromatogram.

of the mass. Fractions H and I had absorbance peaks similar to that seen for the very polar fraction VP [7]. Other fractions had no appreciable absorbance. Based on comparison of retention times to those of standards, the first fractions were much less polar than the later eluting fractions.

The acetone extract of SRM 1649 had a mass distribution similar to that for Elizabeth, as indicated in Table II. Blank-corrected mass recovery was 98%. Absorbance chromatograms at 205 nm are shown in Fig. 2. The total extract mass separated into three broad groups, centered on fractions B, D and H. The mass distribution was similar to that observed for Elizabeth, but the chromatograms did not show much absorbance for the least polar mass peak (B).

The capacity limit of single 500 mg cyanopropyl-bonded silica columns was determined to be between 5.7 and 6.2 mg for the Elizabeth extract. The visually-determined integrity of the fractionation procedure was beginning to deteriorate at 6.2 mg, and no heavier loadings were attempted.

After the acetone extracts of ambient particulate matter had been fractionated and analyzed, the procedure was tested with a standard mixture containing polar aromatic compounds. Compounds were selected on the basis of their polarity and the possiblity of their presence in ambient particulate matter. They also had to have appreciable UV absorbance or fluoresence. Included in the mixture was a compound recently identified as a potent mutagen in dichloromethane extracts of ambient air particles, 2-nitro-6H-dibenzo[*b*,*d*]pyran-6-one [16]. All compounds except 5-nitrovanillin were eluted in fractions A and B. Less than 1% of the applied

TABLE II

FRACTIONATION OF THE ACETONE EXTRACT OF AMBIENT PARTICLES-SRM 1649

Fraction	Solvent	Color	Intensity[a]	Mass recovery, %[b]
A	Hexane	Clear	0	6.8
B	25% $MeCl_2$	Pale	1	8.9
C	25% $MeCl_2$	Clear	0	2.9
D	$MeCl_2$	Faint	0.5	10.3
E	$MeCl_2$	Clear	0	6.7
F	2% MeOH	Clear	0	2.8
G	5% MeOH	Light yellow	2	13.1
H	15% MeOH	Yellow	4	26.2
I	40% MeOH	Pale	1	13.3
J	MeOH	Clear	0	8.8
Total recovery				98

[a] Visual estimate; a value of 4 was arbitrarily assigned to the fraction with the most intense color.
[b] Blank-corrected recovery, %.

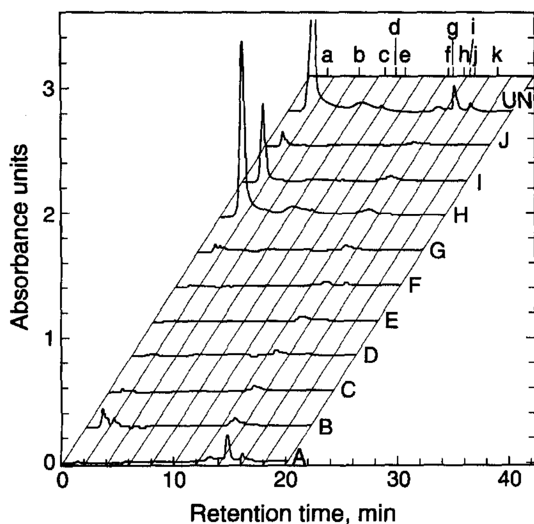

Fig. 2. Reversed-phase chromatograms of the acetone extract of SRM 1649 ambient particles. The ordinate shows absorbance at 205 nm in milliabsorbance units. The top chromatogram indicates the retention times for the standard compounds: a = 5-nitrovanillin; b = vanillin; c = 2-naphthoic acid; d = 1,4-dihydroxynaphthalene; e = 5-nitroquinoline; f = 2-nitro-6H-dibenzo[*b*,*d*]pyran-6-one; g = 2-nitronaphthalene; h = 5,6-benzoquinoline; i = acridine; j = 1-pyrenecarboxaldehyde, and k = dioctylphthalate. The unfractionated extract is labelled as UN; fractions are identified by capital letters at the right of each chromatogram.

mass of any compound was found in any other fraction, with typically at least two thirds of the mass of each compound found in fraction A. 5-Nitrovanillin was not recovered from any of the ten fractions in either attempt. The recovery data from the evaporation and fractionation procedures are given in Table III. Compounds are listed in order of increasing retention time (decreasing polarity) on the reversed-phase HPLC column.

Six compounds showed substantial losses in the evaporation step: three of the four nitro compounds, plus vanillin, 2-naphthoic acid and 1,4-dihydroxynaphthalene. Four are naphthalene derivatives which are fairly volatile; the other two are benzene derivatives with multiple polar groups. These losses were reflected in lower than average fractionation recoveries. The losses were more severe in the first fractionation. The two fractionation experiments differed in whether or not the column was air-dried prior to elution and the amounts of applied material. Low applied masses and a degenerating guard column in the HPLC led to no detection of the first four compounds in the first fractionation. The second fractionation used about four times the mass used in the first fractionation, and somewhat higher recoveries were obtained overall.

TABLE III

EVAPORATION AND FRACTIONATION DATA FOR STANDARD COMPOUNDS

Compound	Intitial mass (μg)			Recovery (%)		
	Evap.[a]	Frac. 1[b]	Frac. 2[c]	Evap.[a]	Frac. 1[b]	Frac. 2[c]
5-Nitrovanillin	4.6	4.6	18.5	71	–	–
Vanillin	4.2	4.2	17.5	39	–	81
2-Naphthoic acid	0.87	0.87	3.5	57	–	82
1,4-Dihydroxynaphthalene	1.6	1.6	6.5	27	–	59
5-Nitroquinoline	4.6	4.6	4.6	60	66	80
2-Nitro-6H-dibenzo[*b*,*d*]pyran-6-one	1.4	1.4	5.4	115	86	91
2-Nitronaphthalene	1.8	1.8	7.1	52	106[d]	78
5,6-Benzoquinoline	1.8	1.8	7.2	112	67	90
Acridine	1.9	1.9	7.7	89	72	90
1-Pyrenecarboxaldehyde	1.6	1.6	6.4	97	52[d]	90
Dioctylphthalate	3.8	3.8	15.2	117	105	96.3
Average ± S.D.				76 ± 32	79 ± 21	84 ± 10

[a] Standard mixture was evaporated to dryness in a water bath and reconstituted in methanol.

[b] Standard mixture was reconstituted in *n*-hexane, dichloromethane and methanol, and fractionated. Column was air dried before elution, as was done for extracts of ambient particles.

[c] Standard mixture was reconstituted in *n*-hexane, dichloromethane and methanol and fractionated without column drying before elution.

[d] These values are less certain because of the condition of the HPLC column.

The averaged recovery results for the other compounds are statistically indistinguishable for the two experiments. The effect of air-drying does not appear to be significant. Losses of the standard compunds appear to be due largely to evaporative losses rather than irreversible adsorption or reaction on the cyanopropyl-bonded silica of the SPE column.

A mixture of the Elizabeth acetone extract and the standard compounds was also fractionated and analyzed by HPLC. The resulting absorbance chromatograms resembled the sum of the independent chromatograms; *i.e.*, there was no apparent interaction between the two sample types. Retention times and recoveries were not substantially changed.

DISCUSSION AND CONCLUSIONS

Cyanopropyl-bonded silica SPE columns proved capable of fractionating mg quantities of acetone extracts of airborne particulate matter with good recovery of mass without addition of water or buffer salts. When eluted using normal-phase chromatography, with solvent mixtures of increasing polarity, the resultant fractions increased in polarity, as judged by HPLC analysis. This is consistent with the results of fractionating pyridine extracts of coal using the same type of SPE columns [11].

The observation that the acetone extracts of ambient particles redissolved in methanol can be explained by noting that methanol is more polar than acetone. Fractionation of the acetone extract has been possible because of the selective elution of components of the material from the cyano SPE adsorbent in a series of solvent mixtures, most of which are much less polar than the extraction solvent, acetone. Since the parent pollution particles had already been extracted with dichloromethane, this result may appear surprising. However, the original two-step extraction process had dissolved species which may have been strongly adsorbed onto the particulate matrix. Once desorbed from that matrix they may have been more soluble in solvent mixtures of lower polarity than they were when sorbed onto the matrix. We found that the ten-fraction separation was only possible when *n*-hexane, a non-polar solvent, was used, along with dichloromethane, to decrease the polarity of the ambient samples and allow adsorption of the polar components onto the cyanopropyl matrix before fractionation could begin. A stronger adsorbent such as non-bonded silica would probably have irreversibly adsorbed the species of interest, perhaps paralleling the earlier adsorption of the polar species to their parent particulate matrix.

The standard compounds tested in this study were apparently less polar or more soluble in *n*-hexane and dichloromethane than the acetone extracts of ambient particulate matter for which the technique has been developed. All the standards eluted before the bulk of the mass of either extract of airborne particles. An earlier study using preparative HPLC with a cyano column [12] found vanillin in the fourth of seven fractions of increasing polarity. (In that study the most polar fractions were not extensively characterized due to the limited recovery of very polar material from gas chromatographic columns.) Vanillin was one of the two most polar compounds tested here, as judged from reversed-phase HPLC retention times. Non-polar and moderately polar species such as vanillin and the nitrated low-molecular-mass polycyclic aromatic hydrocarbons would have been removed from the ambient particles during the first extraction step in dichloromethane.

The technique reported here is easy, fast and inexpensive when compared to fractionation by preparative-scale HPLC. Mass distributions for both acetone extracts of ambient particles and a mixture of polar standard compounds were quite reproducible. The cyano SPE columns showed no evidence for destruction or irreversible adsorption of components in the polar extracts of urban airborne particulate matter.

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REFERENCES

1 J. M. Daisey, R. J. Hershman and T. J. Kneip, *Atmos. Environ.*, 16 (1982) 2161–2168.
2 J. M. Daisey, in P. J. Lioy and J. M. Daisey (Editors), *Toxic Air Pollution, A Comprehensive Study of Non-criteria Air Pollutants*, Lewis, Chelsea, 1987, pp 47–65.
3 J. M. Daisey, T. J. Kneip, I. Hawryluk and F. Mukai, *Environ. Sci. Technol.*, 14 (1980) 1487–1490.
4 T. B. Atherholt, T. B. McGarrity, J. B. Louis, L. J. McGeorge, P. J. Lioy, J. M. Daisey, A. Greenberg and F. Darack, in M. D. Waters, S. S. Sandhu, J. Lewtas, L. Claxton, G. Strauss and S. Nesnow (Editors), *Short-Term Bioassays in the Analysis of Complex Environmental Mixtures IV*, Plenum, New York, 1985, pp. 211–231.
5 M. Zinbo, D. Schuetzle, D. H. Hsieh, N. Y. Kado, J. M. Daisey and L. A. Gundel, *Anal. Sci.*, 8 (1992) 461–468.
6 Y. Yokouchi and Y. Ambe, *Atmos. Environ.*, 20 (1992) 1727–1734.
7 L. A. Gundel, J. M. Daisey, L. R. F. Carvalho, N. Y. Kado and D. Schuetzle, *Environ. Sci. Technol.*, (1992) submitted.
8 A. Sicherer-Roetman, M. Ramlal, C. E. Voogd and H. J. Th. Bloemen, *Atmos. Environ.*, 22 (1988) 2803–2808.
9 A. L. Lafleur, A. G. Braun, P. A. Monchamp and E. F. Plummer, *Anal. Chem.*, 58 (1986) 568–572.
10 M. Zeif and R. Kiser, *Am. Lab.*, January (1990) 70-83.
11 A. L. Lafleur and Y. Nakagawa, *Fuel*, 68 (1989) 741–752.
12 T. J. Mast, D. P. H. Hsieh and J. N. Seiber, *Environ. Sci. Technol.*, 18 (1984) 338–348.
13 J. C. Crowley, S. Siggia and P. C. Uden, *Anal. Chem.* 52 (1980) 1224–1228.
14 P. J. Lioy and J. M. Daisey (Editors), *Toxic Air Pollution, A Comprehensive Study of Non-criteria Air Pollutants*, Lewis, Chelsea, 1987.
15 J. M. Daisey, J. D. Spengler and P. Kaarakka, *Environ. Intern.*, 15 (1989) 435–442.
16 D. Helmig, J. Arey, W. P. Harger, R. Atkinson and J. Lopez-Cancio, *Environ. Sci. Technol.*, 26 (1992) 622–624.