



## Project Summary

# Sampling and Analysis for High-Molecular-Weight Polar Organic Compounds

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**This report gives results of preliminary investigations into the determination of high-molecular-weight polar organic compounds from wood combustion residues. It is intended as a reference to be used by laboratories that are developing methods for the identification and quantification of high-molecular-weight compounds.**

***This Project Summary was developed by EPA's Air and Energy Engineering Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).***

### Introduction

No universal analytical method currently exists in the field of trace organic analysis that covers the more polar, less volatile components of environmental samples. While capillary gas-chromatographic methods may be sufficient for substances that are relatively volatile, there remain many large and polar molecules that have increasing environmental significance. With increasing molecular weight, the number of isomeric compounds to be resolved also increases proportionally. The methods for their determination have not yet been fully developed. Although high-performance liquid chromatography (HPLC) is capable of handling such large and polar molecules, improvements in the applications of this technique will be required for the characterization of high-molecular-weight fractions.

The proposed HPLC method is capable of giving functional-group class separation for semipreparative sample fractionation, and it may prove to be a useful analytical technique for the more polar constituents (pyrrolic, -OH, -COOH, and -NH<sub>2</sub> substituted). But these compounds constitute only about 40% (w/w) of the sample analyzed in this study. About 36% of the sample was judged to contain less polar compounds (carbonyl, nitro, and aza compounds). The remaining 24% were non-polar compounds (hexane extractables). Unfortunately, the method did not distinguish between the alkyl- and benzyl-substituted proton-donating compounds and the nonionizable compounds. It appears that the higher the molecular weight, the greater the alkyl substitution; or the more ring conjugation present, the more inadequate the method becomes as an analytical technique.

The objective of this task was to develop methods for determining high-molecular-weight polar organic compounds derived from coal, synfuel, and wood combustion. The existing methodology was reviewed, and the analytical methodology was developed leading to the separation of as many of the high-molecular-weight species as possible in residues from wood combustion.

The proposed method was expected to be based on initial separation by liquid chromatography, which included such methods as liquid-solid chromatography, gel-permeation chromatography, or reversed-phase liquid chromatography. The use of the pro-



posed method resulted in major sub-fractions and, ultimately, fractions containing fewer compounds. Qualitative analysis was expected to be based on a combination of techniques.

Fast atom bombardment/mass spectrometry (FAB/MS) and direct-insertion-probe/mass spectrometry (DIP/MS) were used to characterize fractions. The samples were also screened by Fourier-transform infrared spectroscopy (FTIR).

### Open-Column Liquid Chromatography

Initial separation of the sample into functional-group classes was attempted using a silica-gel open-column liquid chromatography procedure. The four fractionation schemes progress from nonpolar (hexane) solvents to very polar (methanol and dimethyl sulfoxide) solvents in attempts to separate the woodburn samples into functional group classes. Methylene chloride was a moderately polar solvent used in combination with the nonpolar and very polar solvents.

### HPLC Instrumentation

The HPLC system (Model 1090A) from Hewlett-Packard (Palo Alto, CA) consisted of a binary-gradient solvent-delivery system, diode-array detector, automatic liquid sampler, and a variable-column injector.

### HPLC Chromatographic Methods

1. Column: Waters  $\mu$ -Styragel, 100 Å, 300 × 7.8-mm ID  
Solvent: Tetrahydrofuran  
Flow: 1 mL/min, isocratic
2. Column: ASI Ultragel, 100 Å, 250 × 7.8-mm ID  
Solvent: Tetrahydrofuran  
Flow: 1 mL/min, isocratic
3. Column 1: Waters  $\mu$ -Styragel, 100 Å, 300 × 7.8-mm ID  
Column 2: ASI Ultragel, 100 Å, 250 × 7.8-mm ID  
Solvent: Tetrahydrofuran  
Flow: 1 mL/min, isocratic
4. Column: Whatman Partisil, ODS-3, 10  $\mu$ m, 250 × 4.6-mm ID  
Solvent A: Methanol  
Solvent B: Methylene Chloride  
Flow: 1 mL/min, gradient  
Program: 0% B to 100% B in 40 min.

5. Column: Waters  $\mu$ -Bondapak-NH<sub>2</sub>, 10  $\mu$ m, 300 × 3.9-mm ID  
Solvent: n-Heptane  
Flow: 0.5 mL/min, isocratic
  6. Column: Waters  $\mu$ -Porasil-Si, 10  $\mu$ m, 300 × 3.9-mm ID  
Solvent A: Carbon Tetrachloride  
Solvent B: Dimethyl Sulfoxide  
Flow: 1 mL/min, isocratic at 0.1%, 1.0%, 10%, or 25% B
  7. Column: Waters  $\mu$ -Porasil-Si, 10  $\mu$ m, 300 × 3.9-mm ID  
Solvent A: Carbon Tetrachloride  
Solvent B: Dimethyl Sulfoxide  
Flow: 1 mL/min gradient  
Program: 0% B to 1% B in 10 min, then to 10% B in 10 min
  8. Column: Chromanetics Spherisorb-Si, 5  $\mu$ m, 250 × 4.6-mm ID  
Solvent A: Carbon Tetrachloride  
Solvent B: Dimethyl Sulfoxide  
Flow: 1 mL/min, isocratic
- Method option:  
A = 25% B  
B = 10% B  
C = 1.0% B  
D = 0.1% B

### Initial Sample Characterization

Four woodburn samples were received from EPA for the analysis of high-molecular-weight polar organic compounds:

243B	104.08 mg
243C	100.13 mg
233C	99.65 mg
237D	99.34 mg

Samples 243B and 243C appeared to be duplicates, as did Samples 233C and 237D. All were black, tarry substances coated on the walls of the sample vials. The original samples were submitted for EI/MS, FAB/MS, and FTIR characterization.

FTIR samples were dissolved in methanol and analyzed as a KBr pellet. The spectra obtained on a Nicolet MX-IE in this manner were not intense enough to identify functional groups. The samples should be examined using a higher concentration in the KBr pellet or as a film on a salt plate. However, Samples 243B and 243C appear to have similar

spectra. Samples 233C and 237D also have similar FTIR spectra.

The EI mass spectra obtained with a Varian Mat 311A mass spectrometer showed peaks only below m/z 500. The EI/MS obtained in this manner would not be useful for analysis of higher-molecular-weight polar compounds.

FAB/MS was obtained with a Varian Mat 311A mass spectrometer. Peaks up to m/z = 1500 were observed in the FAB/MS analysis; however, these spectra were very complex. Some representative peaks for the original samples from the FAB/MS analysis are listed in Table 1.

### Open-Column Liquid Chromatography

The spectra obtained by FTIR, EI/MS, and FAB/MS of the samples indicated many compounds of different molecular weights and functional groups. Open-column liquid chromatography may be used to separate materials according to functional groups. Several fractionation schemes were developed in attempts to separate the woodburn samples into less complex fractions.

The open-column liquid chromatography fractionation schemes yielded residues yellow to brown in color. The FAB/MS of these residues contained predominantly low-mass ions. However, some peaks were evident up to m/z 1500. Table 2 gives representative m/z values for three polar fractions of Sample 243B.

### Direct-Insertion-Probe/Mass Spectrometry Results from Scheme 4

An unfractionated portion of Sample 233C and Fractions 1, 2, 3, 4, 5, and 7 of LC fractionation Scheme 4 were analyzed using direct-insertion-probe/mass spectrometry (DIP/MS). All of the Fraction 6 sample was placed in glycerol for FAB/MS and thus was not analyzed by DIP/MS. Between 10 and 50  $\mu$ g of each sample, excluding solvent, was placed in capillary-glass sampling tubes, the solvent evaporated, and the residue analyzed. Representative m/z values for the original sample from the DIP/MS analysis and the individual fractions are summarized in Table 3. The peaks at m/z 167 and 181 may be indicative of carbazole and carbazole derivatives. These polar polynuclear aromatic hydrocarbons (PAHs) may be present in the sample. Further work will better characterize the fractions and will possibly identify more compounds.

**Table 1.** Representative Significant *m/z* Values and Approximate Relative Intensities by FAB/MS Analysis<sup>a</sup>

Sample 243B	Sample 233C	Sample 243C	Sample 237D
<i>m/z</i>	<i>m/z</i>	<i>m/z</i>	<i>m/z</i>
300(10) <sup>b</sup>	304(15)	387(5)	300(15)
304(10)	308(15)	398(5)	310(15)
316(5)	310(12)	973(7)	314(15)
335(5)	412(7)		325(10)
360(5)	414(5)		338(10)
380(4)	425(5)		342(10)
393(4)	480(4)		350(8)
395(4)	503(3)		353(8)
398(4)	550(3)		365(8)
400(4)	592(3)		381(8)
412(3)	642(2)		398(8)
415(3)			413(8)
418(3)			430(5)
436(3)			438(5)
			497(3)

<sup>a</sup>Many peaks were seen at relative intensities of 2 to 3% above *m/z* 1000.

<sup>b</sup>Approximate percent relative intensities given in parentheses.

**Table 2.** Representative Significant *m/z* Values for Approximate Relative Intensities by Positive and Negative FAB/MS Analysis for Sample 243B

Fraction 5		Fraction 6		Fraction 7
Positive	Negative	Positive	Negative	Positive
343 <sup>b</sup> (10) <sup>a</sup>	297 <sup>b</sup> (10)	429 <sup>b</sup> (15)	297 <sup>b</sup> (8)	431(70)
345 <sup>b</sup> (30)	299 <sup>b</sup> (35)	431 <sup>b</sup> (30)	294 <sup>b</sup> (8)	445(50)
347 <sup>b</sup> (15)	301 <sup>b</sup> (25)	433 <sup>b</sup> (20)	301 <sup>b</sup> (8)	463(30)
435 <sup>b</sup> (5)	392(5)	521 <sup>b</sup> (7)		486(20)
437 <sup>b</sup> (12)	416(4)	523 <sup>b</sup> (10)		523(20)
439 <sup>b</sup> (10)	498(3)	525 <sup>b</sup> (8)		548(15)
444(7)		530(5)		585(15)
446(7)		540(5)		598(10)
461(7)		545(5)		650(8)
485(5)		556(5)		684(8)
506(5)		558(5)		715(7)
525(5)		560(5)		795(5)
667(4)		562(5)		803(4)
722(3)		582(5)		820(4)
740(3)		584(4)		846(4)
853(3)		588(4)		873(4)
		647(4)		890(4)
		674(3)		897(4)
		704(3)		925(3)
		752(3)		1010(3)
		894(3)		
		1002(3)		

<sup>a</sup>Approximate percent relative intensities given in parentheses.

<sup>b</sup>These *m/z*'s have been identified as abietic acid, or derivatives of abietic acid (mol wt 302.4), a component of wood rosin.

## HPLC Fractionation

The first approach was to fractionate the sample by molecular weight on a gel-permeation column (Waters  $\mu$ -Styragel, 100 Å, 300 × 7.8-mm ID) and then to analyze each subfraction on a normal-phase column (Waters  $\mu$ -Porasil-Si, 10  $\mu$ m, 300 × 3.9-mm ID). Because one gel-permeation column did not provide adequate separation of the original sample matrix for fractionation, a second GPC column (AS1 ultragel, 100 Å, 250 × 7.8-mm ID) was used in series with the  $\mu$ -Styragel column. Comparing the original sample with standard mixtures of polystyrene at known molecular-weight ranges showed that the sample was composed of polystyrenes with molecular weights ranging from 500 to 1000.

The Waters  $\mu$ -Porasil column was used to investigate the elution order with selected model compounds (pyridine, phenol, aniline, quinoline, carbazole, and dibenzo(a,j)acridine) at various ratios of the additive (DMSO) to the eluent base (CCl<sub>4</sub>) with only marginal success. Peaks within similar compound classes were not resolved adequately. In addition, the less polar groups overlapped badly. An attempt was made to develop a long gradient program, adjusting the amount of additive to base eluent. But as the amount of additive was increased, the amount of background sharply increased. Even though the UV cutoff of DMSO is 268 nm, measurement at 276 to 300 nm is drastically affected, especially above the 5% level of DMSO.

The proposed chromatographic system (Method 8) is capable of giving sufficient class separation for semipreparative sample fractionation. And it may prove to be a useful analytical technique for the more polar constituents (pyrrolic, -OH, -COOH, and -NH<sub>2</sub> substituted). But these compounds constitute only about 40% (w/w) of the woodburn Sample 233C. About 36% of the sample was judged to contain less polar, nonionizable compounds (carbonyl, nitro, and aza compounds). And the remaining 24% were nonpolar compounds (hexane extractables). Unfortunately, it was impossible to distinguish between the alkyl- and benzyl-substituted proton-donating compounds and the nonionizable compounds. As a result, the higher the molecular weight, the greater the alkyl substitution, or the more ring conjugation present, the more inadequate the

method becomes as an analytical technique.

However, the two methods (open-column fractionation followed by normal-phase chromatography) provide a good basis for the analysis of the more polar constituents in environmental samples. More success is expected in the resolution and identification when the less polar low-molecular-weight portions of the sample are removed prior to the proposed separation techniques.

The two methods seem to be a good starting point for the fractionation and analysis of the more polar constituents. But more work is needed to handle the less polar portion of the sample.

**Conclusions**

Separation by open-column liquid chromatography is not entirely satisfactory. Low-molecular-weight compounds need to be removed before separation of the woodburn sample into functional groups.

FAB/MS studies show that the spectra contain ions at almost every m/z to 1500, but the interpretation of the spec-

**Table 3.** Representative m/z Values for DIP/MS Analysis for Woodburn Sample 233C

Original Sample	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 7
m/z	m/z	m/z	m/z	m/z	m/z	m/z
167(100) <sup>a</sup>	167(100)	163(72)	167(100)	167(100)	163(80)	161(80)
181(45)	181(50)	167(70)	181(50)	181(50)	167(100)	178(100)
189(45)	194(50)	181(100)	402(2)	402(5)	181(50)	273(10)
368(10)	197(50)	197(48)			197(40)	329(20)
446(10)		213(30)			239(30)	346(10)
		256(25)				514(5)

<sup>a</sup>Approximate percent relative intensities given in parentheses.

tra is complicated by the relatively high abundance of low-molecular-weight compounds.

DIP/MS analyses indicate the presence of polar PAHs, but give little information above m/z 500.

The proposed HPLC (Method 8) is capable of giving sufficient class separation for semipreparative sample frac-

tionation. It may prove to be a useful analytical technique for the more polar constituents (pyrrolic, -OH, -COOH, and -NH<sub>2</sub> substituted). But these compounds constitute only about 40% (w/w) of the woodburn Sample 233C. About 36% of the sample was judged to contain less polar compounds (carbonyl, nitro, and aza compounds).

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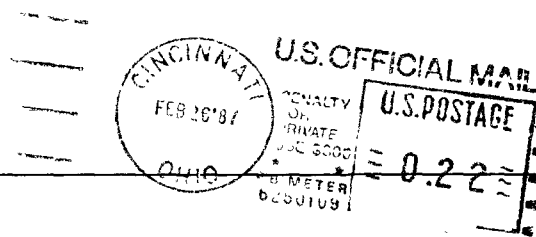
The complete report, entitled "Sampling and Analysis for High-Molecular-Weight Polar Organic Compounds," (Order No. PB 87-119 434/AS; Cost: \$13.95, subject to change) will be available only from:

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