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SUPERCRITICAL-FLUID CHROMATOGRAPHY–MASS SPECTROMETRY OF POLYCYCLIC AROMATIC HYDROCARBONS WITH A SIMPLE CAPILLARY DIRECT INTERFACE

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SUMMARY

Supercritical-fluid chromatography–mass spectrometry (SCF–MS) with carbon dioxide has been used to separate and identify polycyclic aromatic hydrocarbons (PAHs) and heteroatom-containing PAHs with molecular weights up to 532. The capillary direct interface allowed methane chemical ionization (CI) mass spectra to be obtained without modification of a commercially available quadrupole mass spectrometer, and a single instrument can be used for SFC–MS and gas chromatography–MS with a conversion between modes requiring *ca.* 20 min. The interface yields good chromatographic peak shapes, and full-scan spectra were obtained at the low ng level. With selected-ion monitoring, detection limits of *ca.* 25 pg were achieved. SFC–MS analysis of an extract from treated wood and the methane CI mass spectra of 29 standard PAHs and heteroatom-containing PAHs are reported.

INTRODUCTION

Capillary gas chromatography–mass spectrometry (GC–MS) is an extremely powerful technique for the analysis of samples containing polycyclic aromatic hydrocarbons (PAHs) and heteroatom-containing PAHs. However, the ability of conventional GC techniques to elute high-molecular-weight and more polar PAHs is limited by their low volatility. High-performance liquid chromatography (HPLC) has been used for the separation of higher-molecular-weight PAHs, but suffers from lower chromatographic resolution per unit time and, despite intensive attempts by several investigators, the routine coupling of HPLC with MS has proven to be difficult.

Capillary supercritical-fluid chromatography (SFC) is a rapidly developing method for the separation and identification of organic compounds that lack sufficient volatility or thermal stability to be separated by GC. The use of SFC to separate higher-molecular-weight PAHs has been demonstrated, and the coupling of SFC with MS, which has been reported by several investigators, has been the subject of recent reviews[1–9].

In general, the SFC interfaces that have been reported are based on modifications of the existing mass spectrometer interface and/or the ion source, which require

dedication of the mass spectrometer to SFC–MS. For many laboratories, the limited availability of instrumentation and the need to perform conventional GC–MS analyses eliminates the possibility of committing a mass spectrometer to SFC–MS. In such cases, simple SFC–MS interfaces are needed which will allow a single instrument to be interconverted routinely between GC–MS and SFC–MS with a minimum of time. A recent preliminary report[10] has described a simple SFC–MS capillary direct interfacing method which allows a single quadrupole mass spectrometer to be used for GC–MS and SFC–MS with minimal conversion time. In the present report, the use of the simple capillary direct interface to obtain methane chemical ionization (CI) spectra of PAHs and heteratom-containing PAHs under SFC–MS conditions with carbon dioxide is reported.

EXPERIMENTAL

All SFC–MS analyses were performed with a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 5988 GC–MS system. The Model 5890 gas chromatograph that was supplied with the instrument was converted for SFC by installing a Valco (Houston, TX, U.S.A.) Model CI4W HPLC valve, equipped with a 0.5-μl sample loop, above one of the detector holes on the top of the gas chromatograph. (The detector holes are unused, since the interface to the mass spectrometer exits through the side of the GC oven.) The SFC column and the injector splitter assembly entered the oven through one of the detector holes, and were constructed as described by Peadon et al.[11]. A short piece of 10-μm I.D. fused-silica capillary tubing was used to control the split flow to obtain a split ratio of ca. 1:20.

The interfacing of the SFC column to the mass spectrometer was achieved by inserting the column through the transfer line until the restrictor tip of the column extended to ca. 1 mm of the end of the interface probe (i.e., the SFC column was installed in a manner identical to that used for a capillary GC column). An integral "Guthrie" restrictor[12] was used to control column flow at a gas flow-rate of 1–3 ml/min, measured at 200 atm column pressure. No modifications of the commercially supplied GC–MS interface (such as the addition of heating elements at the column outlet) were made. The source temperature and the interface probe temperature were held at 270°C (the same as for the normal GC–MS mode of the instrument).

Since the GC injection ports remained in place, and since the mass spectrometer interface was not modified, converting the instrument from the SFC–MS to the GC–MS mode and back is achieved by simply installing the appropriate capillary column. Chromatographic columns can be changed in the Model 5988 GC–MS system without venting the source. This allows the conversion to be completed in ca. 20 min. The use of the integral restrictor for the SFC column also facilitated conversion, since the restrictor tip is phycically strong enough to allow removal and reinstallation of the SFC column without breaking.

SFC pressure programming was performed using a Lee Scientific (Salt Lake City, UT, U.S.A.) Model 501 pump. All separations were achieved with carbon dioxide as the carrier and a 10-m SB-phenyl-5 column (50 μm I.D., 0.5 μm film thickness), supplied by Lee Scientific.

Methane CI mass spectra (positive ion) were obtained with a source pressure (measured in the ion volume) of ca. 0.3 Torr when the SFC column pressure was 80
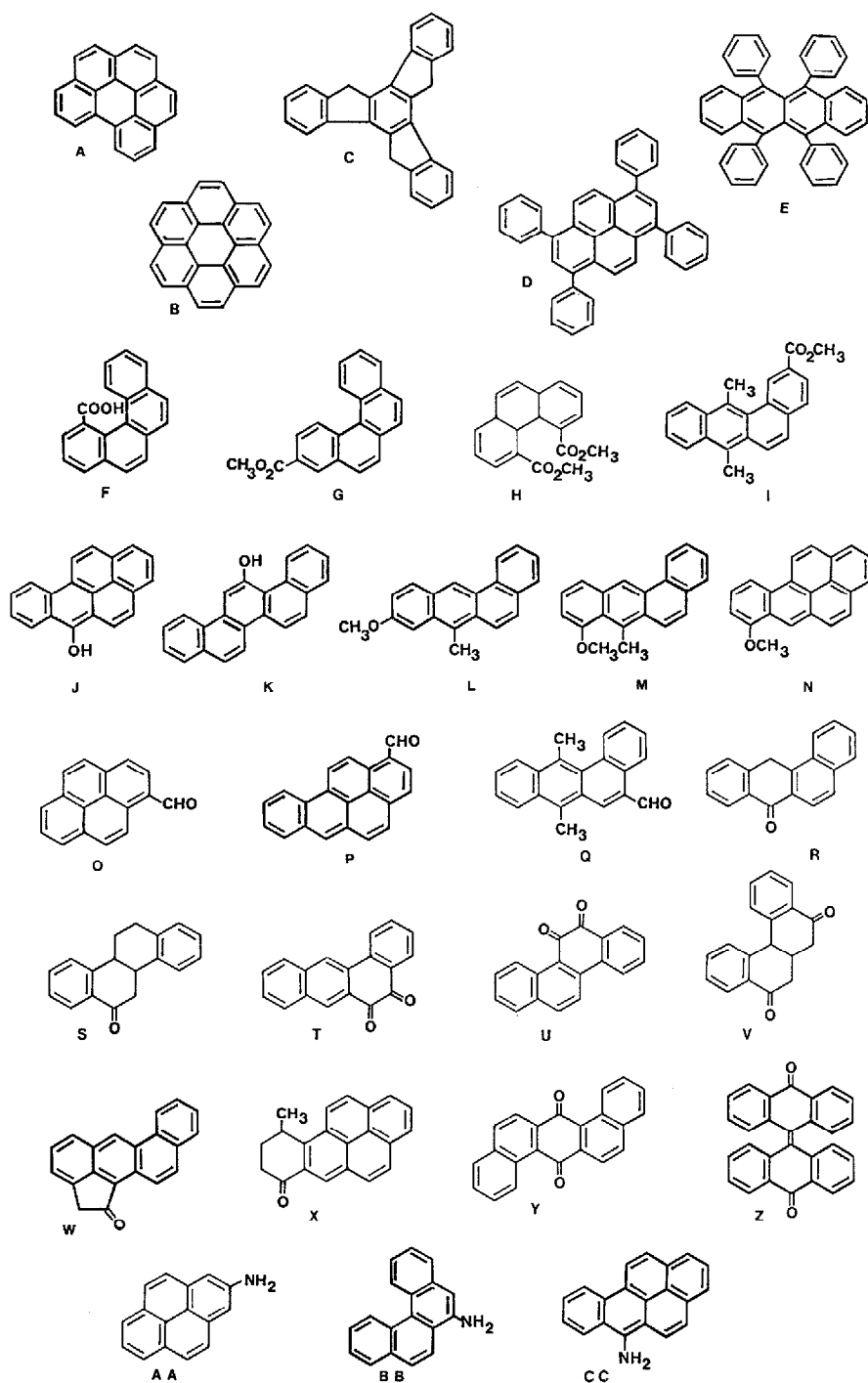
Fig. 1. Structures of the standard PAHs and functionalized PAHs. The letters under each structure correspond to the names and spectra given in Table I.

atm. (Source pressure increased to *ca.* 0.8 Torr during pressure programming up to 400 atm.) Tuning of the mass spectrometer source parameters was performed with polydimethylsiloxane, which was introduced using the direct insertion probe[13] and maximizing the intensity of the ions which occurred at 221, 369, 443, and 591 a.m.u. The ionizing voltage was typically 100 eV. Scan rate was *ca.* 450 a.m.u./s with a typical multiplier voltage of 2700. Standard PAHs and functionalized PAHs were obtained from Aldrich (Milwaukee, WI, U.S.A.) and dissolved in either chloroform or chlorobenzene to a concentration of *ca.* 1 mg/ml for SFC–MS analysis. The structures of the individual standards are shown in Fig. 1.

RESULTS AND DISCUSSION

During the initial development of the capillary direct interface, there was concern that the high pressure of carbon dioxide in the CI source during the SFC separation would produce intense background ions that could interfere with obtaining mass spectra in the lower mass range. Fig. 2 (top) shows the background ions in the spectrum between 50 and 1000 a.m.u. when the column pressure is 200 atm. Although an intense ion appears at $m/z = 59$ (presumably $[O=C-O-CH_3]^+$), only low-intensity background ions occur throughout the rest of the spectrum. The bottom half of Fig. 2 shows the magnitude of the background total ion current (TIC) when the lower limit of the scan range was raised from 50 a.m.u. to 65, 95, and 160 a.m.u. (with 1000 a.m.u. as the upper scan limit in each case). As shown in Fig. 2, the intensity of the TIC from the background ions decreased by a factor of *ca.* 2 for each increase in the lower scan limit. While the total intensity of background ions was fairly high when $m/z = 59$ was included in the scan range, beginning the scan above the mass of this ion gave an acceptable background. Since preliminary results showed that the PAHs and functionalized PAHs gave no significant low-mass fragments under the methane CI conditions, the remaining mass spectra reported in this study were obtained with a lower scan limit of 160 a.m.u. The resulting background was very low, and no
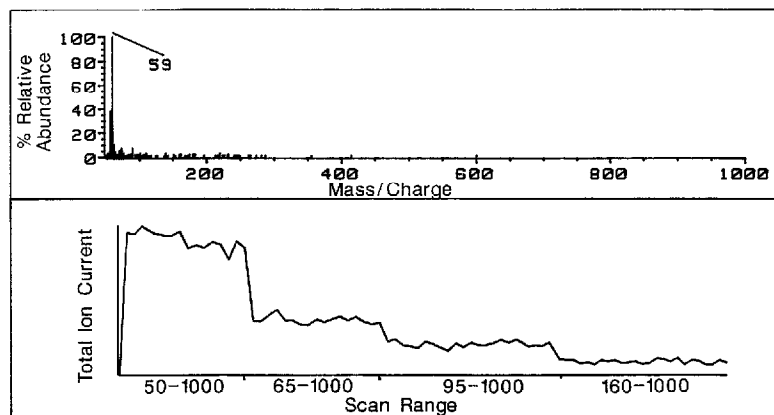

Fig. 2. Background ions in the scan range of 50 to 1000 a.m.u. (top) and the TIC of background ions at different scan ranges (bottom), obtained under SFC–MS conditions with methane chemical ionization. The column pressure was held at 200 atm.
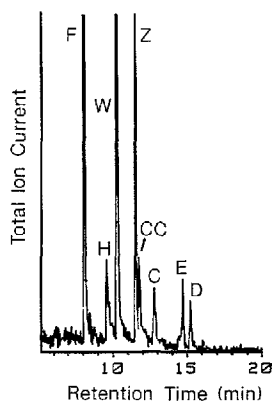
Fig. 3. TIC chromatogram of a mixture of PAHs and functionalized PAHs. The letters on the chromatogram refer to the structures shown in Fig. 1. SFC–MS conditions are given in the text.

background subtraction was used for any of the spectra or chromatograms shown throughout the rest of this report.

The use of the capillary-direct interface to obtain an SFC–MS TIC chromatogram of a mixture of PAHs, oxygenated PAHs, and an amino-PAH is shown in Fig. 3. Approximately 5 to 15 ng of each species were injected at 80 atm and an oven temperature of 125°C, followed by a pressure program of 20 atm/min to 400 atm. A coincident temperature program of 2°C/min to 200°C was also used since slightly better separation of the later-eluting species resulted than when pressure programming was performed at 125°C. The mass spectral scan range was 160 to 1000 a.m.u., and no attempt to reduce the baseline noise level (*i.e.*, background subtraction) was made.

As shown in Fig. 3, the capillary direct interface yielded acceptable chromatographic peak shapes for all of the PAHs, including an underivatized carboxylic acid (benzo[*a*]phenanthrene carboxylic acid, $M = 272$) and the highest-molecular-weight PAH available for this study (5,6,11,12-tetraphenylnaphthacene, $M = 532$). The chromatographic peak shapes shown in Fig. 3 also compare favorably to the peak shapes obtained when the same sample was analyzed under the same chromatographic conditions with a flame ionization detector (not shown).

Representative methane CI mass spectra obtained under the SFC–MS conditions described above are shown in Fig. 4, and the spectra for 29 standard species are summarized in Table I. Each of the spectra was generated with *ca* 50 ng of the test species, injected on-column. With only two exceptions (benzo[*c*]phenanthrene carboxylic acid and dimethyl-4,5-phenanthrenedicarboxylate), all of the standard compounds show the highest intensity ion (base peak) at the pseudomolecular ion $(M + 1)$, formed from the addition of H^+. The adduct ion formed from the addition of $C_2H_5^+$ at $M + 29$ is apparent in all of the spectra. Most of the compounds also show an ion at M^+ which indicates that some charge exchange ionization (from the carbon dioxide) may be occurring under these conditions.

Many of the PAHs and functionalized PAHs show little fragmentation. This might be expected, since the electron impact spectra of such species also generally show little fragmentation. However, several of the compounds give useful fragmentation patterns. For example, each of the esters shows an ion at $M - 31$, corresponding to the
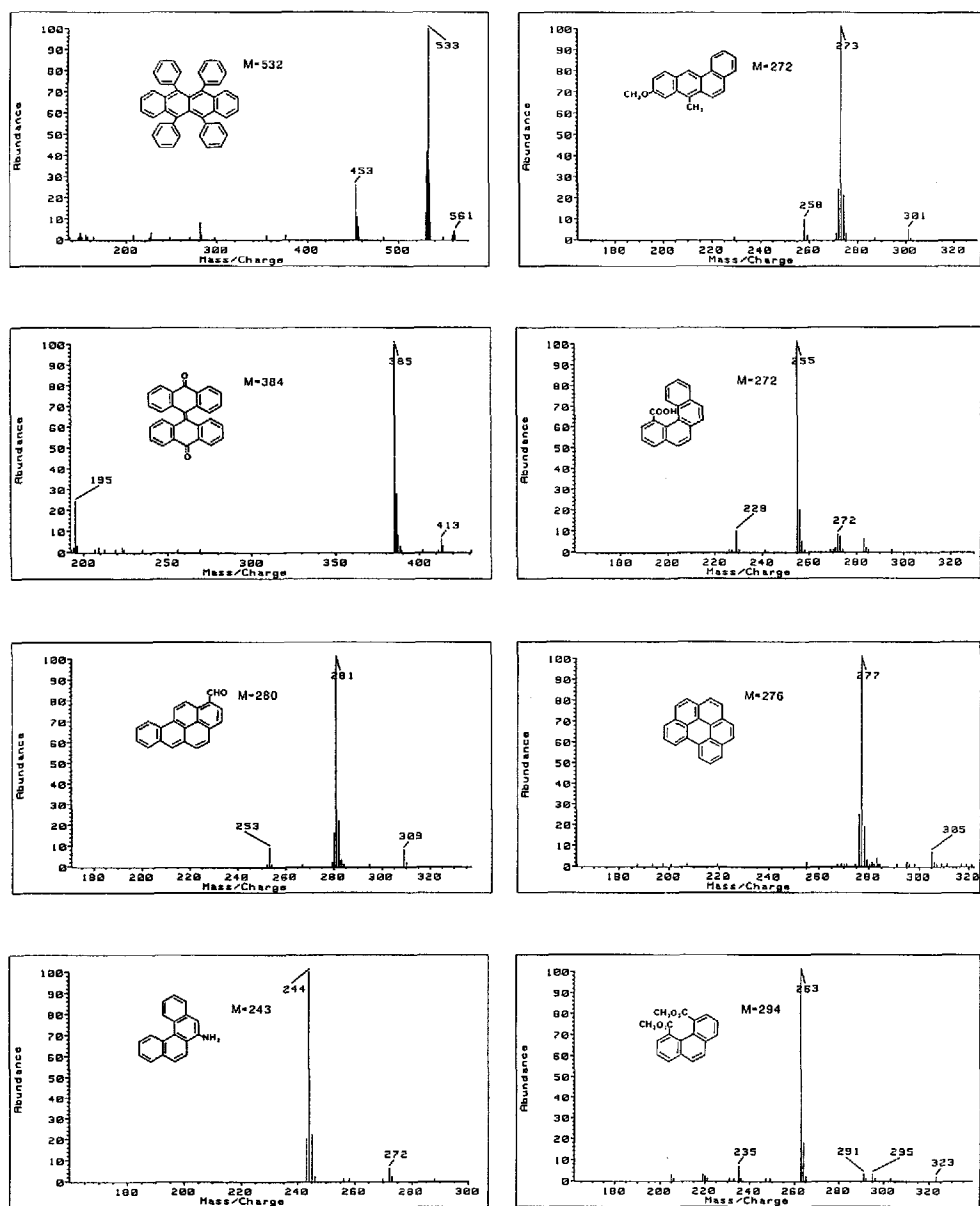
Fig. 4. Representative methane CI mass spectra obtained under SFC–MS conditions.

loss of methanol from the ionized parent. Benzo[c]phenanthrene carboxylic acid shows a base peak at $m/z = 255$ due to the loss of water from the ionized parent. This acid also shows significant M and M + 1 ions as well as an ion at $m/z = 229$, due to the loss of CO_2 from the ionized parent. The diester, dimethyl-4,5-phenanthrenedicarboxylate (Fig. 1H), shows a base peak at $m/z = 263$, due to the loss of methanol from the

TABLE I

SFC–MS METHANE CI MASS SPECTRA OF PAHs AND FUNCTIONALIZED PAHs

| Species[a]		Mol. wt.	Relative intensity (%)[b]				
			M + 1	M + 29	Other ions (mass, intensity)[c]		
PAHs							
A	Benzo[ghi]perylene	276	100	7	276(25)		
B	Coronene	300	100	12	300(31)		
C	Truxene	342	100	8	342(18)	268(9)	269(8)
D	1,3,6,8-Tetraphenylpyrene	506	100	5	506(21)		
E	5,6,11,12-Tetraphenylnaphthacene	532	100	5	532(40)	531(30)	453(27)
					455(12)	530(10)	
O-PAHs							
Acids and esters							
F	Benzo[c]phenanthrene carboxylic acid	272	9	1	255(100)	229(11)	272(10)
G	Methylbenzo[c]phenanthrene-7-carboxylate	286	100	7	286(11)	255(14)	243(9)
H	Dimethyl-4,5-phenanthrene-dicarboxylate	294	4	2	263(100)	235(9)	291(5)
					295(5)		
I	Methyl-1,12-dimethylbenz-[a]anthracene-2-carboxylate	314	100	6	314(20)	283(6)	
Alcohols and ethers							
J	6-Hydroxybenzo[a]pyrene	268	100	8	268(12)		
K	3-Hydroxypicene	294	100	16	294(18)		
L	9-Methoxy-7-methylbenz-[a]anthracene	272	100	5	272(24)	258(11)	
M	8-Methoxy-7-methylbenz-[a]anthracene	272	100	5	272(25)	258(5)	
N	7-Methoxybenzo[a]pyrene	282	100	2	282(28)		
Aldehydes and ketones							
O	4-Pyrenecarboxaldehyde	230	100	9	203(16)		
P	1-Benzo[a]pyrenecarboxaldehyde	280	100	9	280(16)	253(9)	
Q	7,12-Dimethylbenz[a]anthracene-5-carboxaldehyde	284	100	6	284(10)	257(5)	
R	7,12-Dimethylbenz-[a]anthracene-7-one	244	100	5	244(21)	259(6)	
S	Hexahydrochrysene-6-one	248	100	7	207(15)		
T	5,6-Dihydrobenz[a]anthracene-5,6-dione	258	100	2	231(23)		
U	11,12-Dihydrochrysene-11,12-dione	258	100	2	231(14)		
V	Hexahydrobenzo[c]phenanthrene-5,8-dione	262	100	7			
W	Cholanthren-1-one	268	100	8	268(14)	241(12)	
X	7,8,9,10-Tetrahydro-10-methyl-benzo[a]pyrene-7-one	284	100	7	284(12)		
Y	7,14-Dihydrodibenz[a,h]-anthracene-7,14-dione	308	100	8			
Z	Bianthrone	384	100	9	195(26)		
NPAHs							
AA	2-Aminopyrene	217	100	8	217(15)		
BB	6-Aminobenzo[c]phenanthrene	243	100	7	243(24)		
CC	6-Aminobenzo[a]pyrene	267	100	2	267(45)		

[a] The letters refer to the structures shown in Fig. 1. Species names are those used by the supplier.

[b] Each ion with a relative intensity > 5% is reported. Spectra are corrected for ^{13}C contribution.
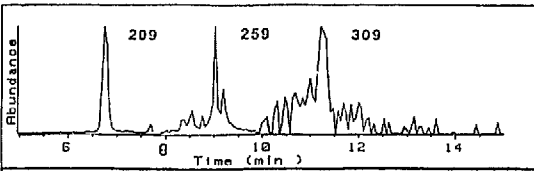
[c] The mass of the ion is followed by its relative intensity in parentheses.
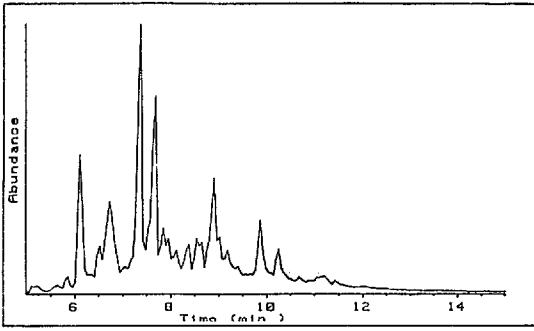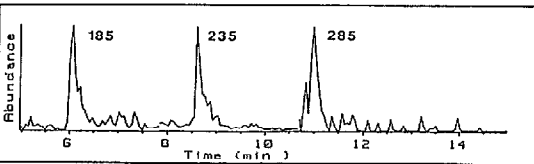
PAHs (M=178,202,228,252,276,278,300,302)
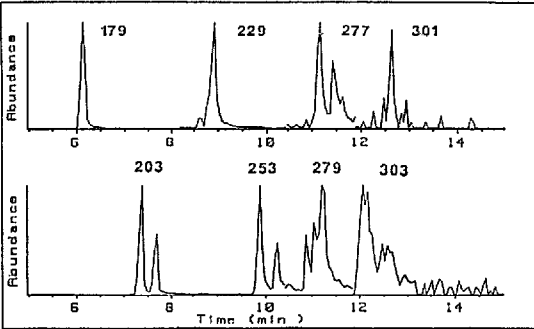
Polycyclic Diones (M=208,258,308)

Polycyclic Thiophenes (M=184,234,284)
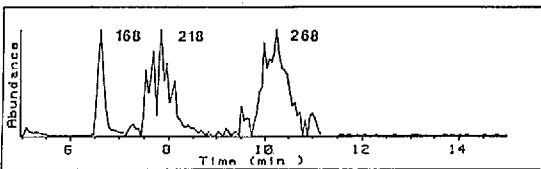

Polycyclic Pyrroles (M=167,217,267)

Fig. 5. SFC–MS analysis of a chloroform extract of a treated wooden utility pole. The TIC chromatogram (top) is shown along with the selected ion plots from the same SFC–MS analysis for the M + 1 ions of PAHs, polycyclic diones, polycyclic thiophenes, and polycyclic pyrroles. SFC–MS conditions are given in the text.
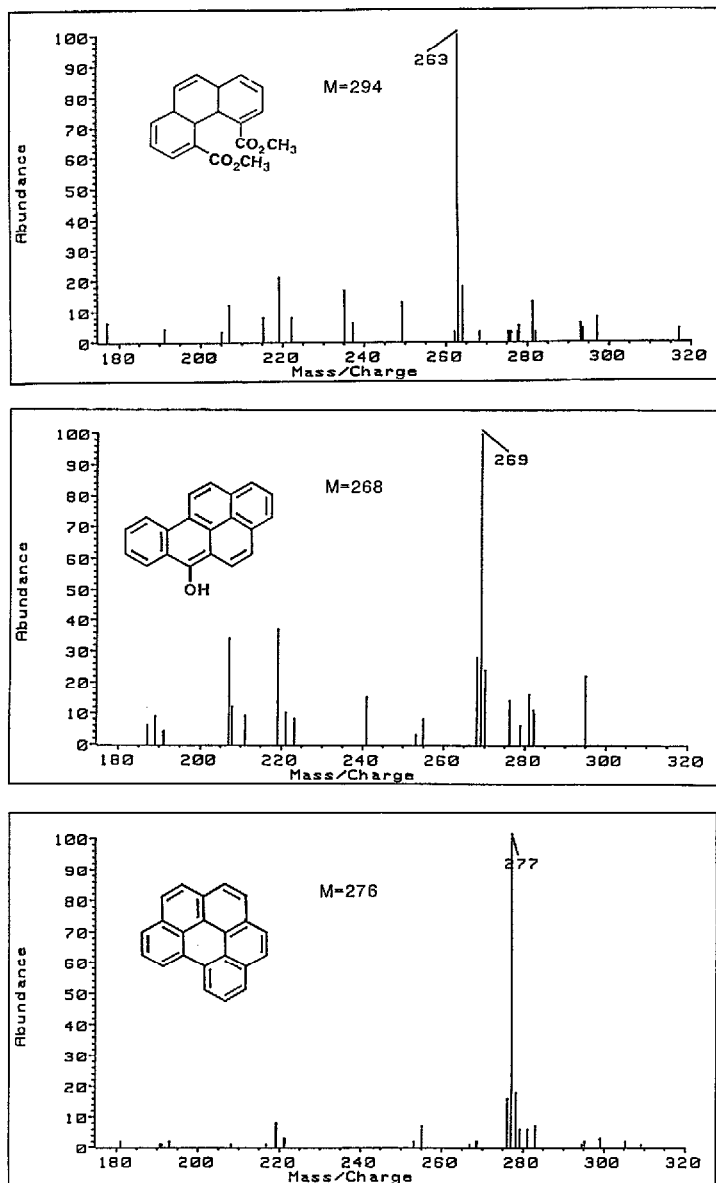
Fig. 6. SFC–MS full-scan (160–1000 a.m.u.) spectra, obtained with 2–3 ng each of dimethyl-4,5-phenanthrenedicarboxylate (H), 6-hydroxybenzo[a]pyrene (J), and benzo[ghi]perylene (A).

ionized parent. Significant ions were also observed at $M + 1$, at the $M + 29$ adduct ($m/z = 323$), as well as at $m/z = 291$, due to the loss of methanol from the adduct ion. Bianthrone (Fig. 1Z) shows a cleavage product ion at $m/z = 195$, while several of the other aldehydes and ketones show fragment ions at $M - 27$, corresponding to the loss of CO from the ionized parent. While the fused-ring PAHs, coronene (Fig. 1B) and benzo[ghi]perylene (Fig. 1A), show no significant fragments (similar to their electron

impact spectra), both the 1,3,6,8-tetraphenylpyrene (Fig. 1D) and the 5,6,11,12-tetraphenylnaphthacene (Fig. 1E) show significant fragment ions.

The SFC–MS analysis of a complex PAH mixture is shown in Fig. 5 by the analysis of a chloroform extract from a treated wooden utility pole. The sample was injected at a pressure of 80 atm, followed by a pressure program of 20 atm/min to 400 atm. The oven temperature was programmed from 125°C to 200°C at 2°C/min. Because of the complexity of the sample, only the major PAHs (molecular weights ranging from 178 to 252) showed distinct chromatographic peaks in the TIC chromatogram, as shown in the top of Fig. 5. However, when the selected ion plots were constructed for the M + 1 ions of several PAHs and heteroatom-containing PAHs, several other species could be tentatively identified by using the same SFC–MS analysis. PAHs in the sample ranged from molecular weights of 178 to 302, polycyclic diones ranged from anthraquinone (M = 208) to dibenzoanthraquinone isomers (M = 308), polycyclic thiophenes ranged from dibenzothiophene (M = 184) to five-fused-ring thiophene isomers (M = 284), and polycyclic pyrroles ranged from carbazole (M = 167) to dibenzocarbazole isomers (M = 267).

The sensitivity achieved by use of the capillary direct interface in the full-scan mode (160–1000 a.m.u.) was investigated by injecting ca. 2–3 ng each of dimethyl-4,5-phenanthrenedicarboxylate, 6-hydroxybenzo[a]pyrene, and benzo[ghi]perylene. (The amount injected into the SFC column was calculated from the measured split ratio, the volume of the sample, and the concentration of the standard solution.) As shown in Fig. 6, the spectra (single scan) contained significant background ions that appear to be random system noise (no background subtraction has been used). However, the most intense ions for each species were easily recognizable and displayed ^{13}C isotope peaks with intensities that agree within ca. 3% of the calculated values, indicating that mass spectral data can be achieved at the ng level in the full-scan mode.

The sensitivity that could be achieved by using selected-ion monitoring (SIM) was also determined by injecting 22–35 pg of the same three species, and monitoring the pseudomolecular ions at M + 1 for 6-hydroxybenzo[a]pyrene and benzo[ghi]perylene and the ion at m/z = 263 for dimethyl-4,5-phenanthrenedicarboxylate. As shown
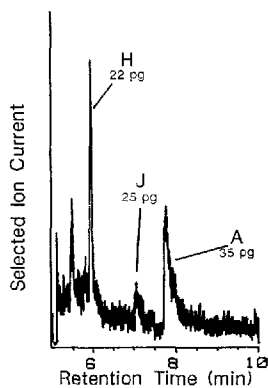

Fig. 7. Selected-ion current chromatogram of 22–35 pg each of dimethyl-4,5-phenanthrenedicarboxylate (H), 6-hydroxybenzo[a]pyrene (J), and benzo[ghi]perylene (A), obtained with SIM SFC–MS analysis. Chromatographic and SIM conditions are given in the text.

by the selected-ion current chromatogram in Fig. 7, each of the three species could be detected at the 22–35 pg level. The results shown in Figs. 6 and 7 demonstrate the ability of the simple capillary direct SFC–MS interface to yield full-scan and SIM sensitivities approaching those normally associated with conventional GC–MS analysis by the use of a quadrupole mass spectrometer operated in the positive-ion mode.

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