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Comparison of Liquid Chromatography/Mass Spectrometry Interfaces for the Analysis of Polycyclic Aromatic Compounds

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Three liquid chromatography/mass spectrometry interfaces were evaluated for their suitability for the analysis of complex mixtures of polycyclic aromatic compounds (PACs). Preliminary qualitative experiments, which used a carbon black extract as test material, confirmed that the moving belt interface is mechanically awkward, is limited with respect to the mobile phase composition which it can tolerate, and is not efficient in detecting the more volatile compounds. For these reasons it was not examined further, although it performed well for larger PACs and provided electron ionization (EI) mass spectra. The particle beam (PB) interface also provides EI spectra, but detection limits are poor (low nanogram range) and calibration curves are nonlinear. Only seven of the 16 PACs targeted for quantification in a complex coal tar reference material could be detected because of the difficulty the PB interface has with the analysis of compounds with very high or very low volatility. The heated pneumatic nebulizer (HPN) interface, which uses atmospheric pressure chemical ionization, produces both molecular ions ($M^{\cdot+}$) and protonated molecules (MH^+) of PACs. Detection limits were in the low picogram range, and calibration curves were linear. Using the HPN interface, 17 target PACs in the coal tar reference material could be detected and quantified within satisfactory agreement with certified values when perdeuterated internal standards were employed.

Polycyclic aromatic compounds (PACs) are potent environmental mutagens and carcinogens, formed from both natural (e.g., biosynthesis and natural combustion) and anthropogenic (e.g., high-temperature combustion) sources.[1] Complex mixtures of these ubiquitous compounds are commonly found in airborne particulates, tobacco smoke, fossil fuels, marine sediments, and food. In addition to health issues, the petroleum and synthetic crude oil industries are also concerned with the interferences that PACs produce in various upgrading processes. In particular, nitrogen-containing PACs are suspected of deactivating and poisoning catalysts during cracking and reforming processes. Plugging problems and reduced heat exchange also arise from the formation of high molecular weight polycyclic aromatic hydrocarbon (PAH) deposits in exchangers, transfer lines, and valves.[2]

The difficulties associated with the characterization of samples containing PAC fractions are due primarily to the large numbers of possible compounds and isomers (which increase with molecular weight). Even for routine qualitative analyses, therefore, methods combining good chromatographic resolving power with selective and sensitive detection are essential for meaningful results to be obtained. Although gas chromatography (GC) possesses high resolving power and can be coupled easily with mass spectrometry (GC/MS), its applicability is restricted by the limited volatility of PACs of higher molecular weights. In addition, the separation selectivity of GC for isomeric PACs is often disappointing. Quantitative analysis of complex matrices for target PACs is an even more demanding task, for which methodology is still being developed or improved. The activities of the U.S. National Institute of Standards and Technology (NIST) in this area have been summarized recently.[3]

Liquid chromatography (LC) has several advantages over GC. Less sample cleanup is required, thermally labile compounds are more easily analyzed since they are not exposed to excessive heat (though this is not a major consideration for PACs), derivatization is usually not necessary, and PACs with high molecular weights may be analyzed because volatility is not an issue for optical detection and is not such a stringent requirement for LC/MS analysis. LC is also an inherently better quantitative method because of the injection volume precision available with fixed loop injectors compared with that for syringe injections with GC. The high chromatographic efficiency available from capillary GC, however, is not currently available from conventional LC. Thus, LC chromatographic peaks are more likely to contain unresolved components, which increases the need for more selective detection methods, such as mass spectrometry. The lower chromatographic efficiency of LC is offset to some degree by the higher separation selectivity made possible through manipulation of the composition of both stationary and mobile phases, exemplified[3-5] by the LC

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(1) Lee, M. L.; Novotny, M. B.; Bartle, K. D. *Analytical Chemistry of Polycyclic Aromatic Compounds*; Academic Press: New York, 1981.
(2) Sullivan, R. F.; Boduszynski, M. M.; Fetzer, J. C. *Energy Fuels* **1989**, *3*, 603–612.
(3) Wise, S. A.; Schantz, M. M.; Benner, B. A., Jr.; Parris, R. M.; Rebbert, R. E.; Sander, L. C.; Koster, B. J.; Chesler, S. N.; May, W. E. *Fresenius J. Anal. Chem.* **1993**, *345*, 325–329.
(4) Sim, P. G.; Boyd, R. K.; Gershey, R. M.; Guevremont, R.; Jamieson, W. D.; Quilliam, M. A.; Gergely, R. J. *Biomed. Environ. Mass Spectrom.* **1987**, *14*, 375–381.

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separation of some PAC isomers which are difficult or impossible to separate by GC.

The main obstacle to routine analytical applications of LC coupled directly to MS (LC/MS) has been the unavailability of rugged and reliable LC/MS interfaces, and this has also been true in the special case of PAC analysis. We have employed the moving belt (MB) interface previously for the analysis of PACs in marine sediments[4,5] and for qualitative characterization of high molecular weight PACs (MW up to 580) in a sample from a coal tar dump site.[6] Severe limitations on the mobile phase composition and flow rates were encountered. In general, solvent gradients were restricted to a low aqueous content and/or low flow rates to ensure that almost all the mobile phase was evaporated before the belt entered the ion source.

This paper describes a comparison of the MB, particle beam (PB), and heated pneumatic nebulizer (HPN) LC/MS interfaces for qualitative and quantitative analyses of PACs using reversed phase LC. Both the MB and PB interfaces were used with electron ionization (EI), while the HPN interface used atmospheric pressure chemical ionization (APCI). The capabilities of the three LC/MS interfaces to provide qualitative information on PACs with a wide range of molecular weights were tested using an extract of carbon black. In evaluations of the interfaces for quantitative analyses, detection limits and linear dynamic ranges were compared using PAC standards, and the concentrations of 17 PACs in the NIST certified coal tar reference material (SRM 1597) are reported for measurements using internal standardization with perdeuterated PAC standards. The objective of the work was to determine whether the advantages of LC/MS over GC/MS (ability to analyze larger PACs and greater separation selectivity) could be implemented in a rugged, reliable methodology with adequate sensitivity and dynamic range.

EXPERIMENTAL SECTION

Materials. HPLC grade acetonitrile and dichloromethane were obtained from BDH Chemicals (BDH Inc., Toronto, Canada) and were used without further purification. Distilled water was passed through a Milli-Q water purification system (Millipore Corp., Bedford, MA) before use. All solvent mixtures are specified as volume/volume ratios. PAC standards from Aldrich (Milwaukee, WI), Supelco (Oakville, Canada), and Anachemia (Montreal, Canada) were used without further purification.

A sample prepared by dichloromethane Soxhlet extraction of carbon black was received as a gift from Dr. J. Fetzer of Chevron Oil. This sample was redissolved in dichloromethane and filtered before use in LC experiments. A complex mixture of PACs from coal tar, standard reference material (SRM) 1597, and a standard solution of 16 priority pollutant PAHs in acetonitrile, SRM 1647, were purchased from the U.S. NIST (Gaithersburg, MD). A solution containing 21 perdeuterated PACs, DPAC-1, developed[7] by the Marine Analytical Chemistry Standards Program (MACSP) of the National Research Council (NRC) of Canada, was used as an internal standard solution for quantification experiments.

For purposes of quantification using internal standards, the DPAC-1 standard solution was spiked at four different levels into the NIST SRM 1647 PAH mixture, in ratios of 1:1, 1:5, 1:10, and 1:15 (v/v). The *exact* amounts of the two solutions mixed were determined by weighings using a 5-place digital balance. The DPAC-1 solution was designed to have *ratios* of analyte concentrations similar to those found in environmental samples,[7] but all 16 analytes in the SRM 1647 solution are at roughly the same concentration. The four spike ratios were therefore chosen to ensure that the concentration ratio of each native and perdeuterated PAH pair was approximately 1:1 in at least one of the mixed solutions. The DPAC-1 internal standard solution was also spiked into the NIST SRM 1597 coal tar mixture in a ratio of 3:1 (v/v). This spike ratio ensured that most of the analyte/internal standard concentration ratios fell within the ranges covered by the DPAC-1/SRM 1647 solutions.

Safety. Since many polycyclic aromatic compounds are mutagenic and carcinogenic, they must be handled with care. Acetonitrile and dichloromethane are toxic, volatile solvents that should be handled in a fume hood. All of these substances are harmful if swallowed, inhaled, or absorbed through the skin.

Liquid Chromatography/Mass Spectrometry. All analyses were performed using a 25 cm × 2.1 mm i.d. column packed with 5 μm Vydac 201TP octadecylsilica (Separations Group, Hesperia, CA) and an HP 1090M chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a variable volume injector/autosampler, a DR5 ternary solvent delivery system, a built-in diode array detector, and an HP7994A data system.

A mobile phase flow rate of 0.2 mL min^{-1} was used. Separations were performed with three different gradient programs: (A) Starting with 100% acetonitrile and held for 15 min, and then linearly programmed to 100% dichloromethane over 40 min and held for a further 15 min. This binary gradient was used in early work on the MB interface; it sacrificed chromatographic resolution of the early-eluting PACs but provided good resolution of the higher molecular weight components without excessive retention times and avoided the use of water, which proved troublesome for the routine use of the MB interface using gradient elution. (B) Starting with 40:60 water/acetonitrile, linearly programmed to 100% acetonitrile over 30 min, held 5 min, and then linearly programmed over 40 min to 100% dichloromethane, with a subsequent 25 min hold prior to recycling the column. This ternary gradient was used for the analysis of the carbon black extract using the PB and HNP interfaces. (C) Starting with 40:60 water/acetonitrile, linearly programmed to 100% acetonitrile over 30 min, held for an additional 30 min before being programmed back to the initial conditions over 5 min. This binary gradient was used for the coal tar SRM with the PB and HNP interfaces.

The MB interface experiments were conducted using a VG Organic 20-250 quadrupole mass spectrometer (Fisons Instruments, Altrincham, U.K.) equipped with a VG MB LC/MS interface. A VG spray deposition device was used to deposit the LC effluent on to the moving polyimide belt while simultaneously evaporating some of the mobile phase. The belt speed was maintained at 1.6 cm/s, and a post-ion source isopropyl alcohol belt wash was employed to avoid memory effects. The electron ionization source was operated at 250 °C, and the belt heater at the tip of the interface, which protrudes into the ion source, was operated with a supply voltage of 0.8 V (belt surface temperature unknown). By adjustment of the intermediate pumping stages, the instrument could be operated at an indicated pressure reading

(5) Quilliam, M. A.; Sim, P. G. *J. Chromatogr. Sci.* **1988**, *26*, 160–167.
(6) Perreault, H.; Ramaley, L.; Sim, P. G.; Benoit, F. M. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 604–610.
(7) Quilliam, M. A.; Hardstaff, W. R.; Anacleto, J. F.; LeBlanc, M. D.; Stergiopoulos, V.; Dick, K. L.; Bowser, M. T.; Curtis, J. M.; Embree, D. J.; Sim, P. G.; Boyd, R. K. *Fresenius J. Anal. Chem.* **1994**, *350*, 109–118.

of 1.5×10^{-5} Torr (read from the ion gauge located on the source housing). This corresponds to pressure conditions inside the EI source such that chemical ionization occurs to only a negligible extent. The nominal electron energy was 70 eV, with a trap current of 100 μA. A VG 11-250 data system was used for instrument control and for data acquisition and processing. Mass spectra were acquired over a range *m/z* 150–600 with a scan cycle time of 3 s.

LC/MS analyses with a PB interface were performed using a Hewlett-Packard instrument comprising a Model 59980A particle beam interface and a Model 5988A quadrupole mass spectrometer. A detailed description of this PB interface can be found elsewhere.[8,9] Ultrahigh purity helium (Liquid Carbonic, Scarborough, Canada) was used as the nebulizing gas at a flow rate of ~1.5 L/min. The temperature of the desolvation chamber was maintained at 45 °C. EI spectra were obtained using a nominal electron energy of 70 eV, with a source temperature of 250 °C. Mass spectra were acquired over a range of *m/z* 100–500, with a scan cycle time of 3 s. Selected ion recording experiments on the NIST-SRM/DPAC-1 quantitation solutions monitored a total of 24 ions in three acquisition periods (8 ions/period, 1 s total cycle time): first retention time period, 0–9.8 min; second period, 9.8–33.5 min; and the last period, 33.5–60 min.

LC/MS analyses with a HPN interface and APCI were performed using an API III triple quadrupole mass spectrometer (SCIEX, Concord, Canada) equipped with an atmospheric pressure ionization (API) source. A detailed description of the HPN interface can be found elsewhere.[10] The corona discharge electrode current was maintained at 3 μA, and high-purity air was used as the nebulizing gas at a flow rate of 1.2 L/min. Full scan mass spectra were acquired over a range of *m/z* 100–500, with total scan cycle times between 2 and 3 s. A Macintosh IIx computer was used for instrument control, data acquisition, and processing. Selected ion recording experiments on the mixed NIST-SRM/DPAC-1 quantitation solutions monitored a total of 48 ions ($M^{\bullet+}$ and MH^+) in six acquisition periods (8 ions/period, 1 s total cycle time): first period, 0–8.8 min; second period, 8.8–10.5 min; third period, 10.5–18 min; fourth period, 18–33.4 min; fifth period, 33.4–46 min; and the last period, 46–60 min.

RESULTS AND DISCUSSION

Qualitative Analysis. The objective of this part of the work was to compare the more common LC/MS interfaces with respect to their suitability for analysis of the PACs of higher molecular weight, which are not amenable to GC/MS. Carbon black is a suitable test sample in this context, since it has been shown[11] that high molecular weight PACs can be extracted from such materials. In our hands, the thermospray interface provided no useful information on PACs and was not investigated further. The electrospray method has been shown by Van Berkel et al.[12,13] to be capable of forming molecular radical cations of PACs, using either charge-transfer complex formation with a suitable electron acceptor or electrochemical oxidation. However, success was strongly dependent on a correct choice of solvent (generally dichloromethane with 0.1% trifluoroacetic acid[12,13]), which is incompatible with gradient elution to achieve efficient LC separations. Moreover, at least in our hands, as applied to both fullerene derivatives and PACs,[14] these electrospray techniques for PACs are neither sufficiently robust nor sensitive at present to provide a useful quantitative analytical method for environmental samples. Accordingly, only the MB, PB, and HPN interfaces were investigated further in the present work.

An early demonstration of LC/MS analysis of a liquefied coal product[15] used a MB interface to confirm identities of the LC peaks. A careful study of the effects of operating parameters on both the LC and MS performance[16] used PAC standards as the test compounds. Analyte volatility was shown[17] to have an effect on both the accuracy and the precision of LC/MS determinations of PACs using the MB interface. More recent work[4–6] from this laboratory has confirmed and extended these earlier findings. In particular, use of the MB interface to provide qualitative profiles of PACs with molecular weights up to 580 was demonstrated.[6] The problem of assigning compound identities to chromatographic peaks was faced[5] in the LC/MS analysis of a marine sediment for PACs. Even when a standard compound is found to provide a good retention time match, it is often difficult to eliminate the possibility that the unknown compound is another isomer. This is, of course, a problem for all LC/MS experiments, not only those using the MB interface. The examination of UV spectra in conjunction with MS data (using the MB interface) was found[5] to help significantly with the problem of peak identification.

The performance of the MB interface in LC/MS profiling of the carbon black extract was consistent with expectations based on past experience with this interface. The total ion current chromatogram (TIC) matched reasonably well the chromatogram obtained using UV detection at 254 nm (not shown), except for the early-eluting (more volatile) components. Because EI was used, fragment ions from compounds of higher molecular weights, particularly alkylated aromatics, can be confused with molecular ions of lower mass compounds, and the complete mass spectra were essential for resolving this ambiguity. Analysis of such spectra indicated that almost all the peaks in the reconstructed ion chromatograms (RICs) represent signals from molecular ions of the PACs. Examples of such spectra and of the RICs can be found elsewhere.[18] However, the performance for relatively involatile and low-abundance compounds of molecular weights up to 424 was gratifying. Note, however, that these LC/MS experiments with the MB interface used the simpler acetonitrile/dichloromethane gradient A (see Experimental Section) since it is more difficult to operate the MB interface routinely with an aqueous gradient (isocratic elution can be handled relatively easily). Also, relatively poor sensitivity for volatile PACs of MW < 200 Da was observed and ascribed to evaporative losses in the

(8) Willoughby, R. C.; Browner, R. F. *Anal. Chem.* **1984**, *56*, 2626–2631.
(9) Winkler, P. C.; Perkins, D. P.; Williams, W. K.; Browner, R. F. *Anal. Chem.* **1988**, *60*, 489–493.
(10) Anacleto, J. F.; Boyd, R. K.; Pleasance, S.; Quilliam, M. A.; Howard, J. B.; Lafleur, A. L.; Makarovsky, Y. *Can. J. Chem.* **1992**, *70*, 2558–2568.
(11) Peaden, P. A.; Lee, M. L.; Hirata, Y.; Novotny, M. *Anal. Chem.* **1980**, *52*, 2268–2271.
(12) Van Berkel, G. J.; McLuckey, S. A.; Glish, G. L. *Anal. Chem.* **1991**, *63*, 2064–2068.
(13) Van Berkel, G. J.; McLuckey, S. A.; Glish, G. L. *Anal. Chem.* **1992**, *64*, 1586–1593.
(14) Anacleto, J. F.; Quilliam, M. A.; Boyd, R. K.; Howard, J. B.; Lafleur, A. L.; Yadav, T. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 229–234.
(15) Dark, W. A.; McFadden, W. H.; Bradford, D. L. *J. Chromatogr. Sci.* **1977**, *15*, 454–460.
(16) Hayes, M. J.; Lankmayer, E. P.; Vouros, P.; Karger, B. L.; McGuire, J. M. *Anal. Chem.* **1983**, *55*, 1745–1752.
(17) Krost, K. J. *Anal. Chem.* **1985**, *57*, 763–765.
(18) Anacleto, J. F. Chromatographic–Mass Spectrometric Analysis of Polycyclic Aromatic Compounds. Ph.D. Thesis, Dalhousie University, Halifax, Canada, 1993.

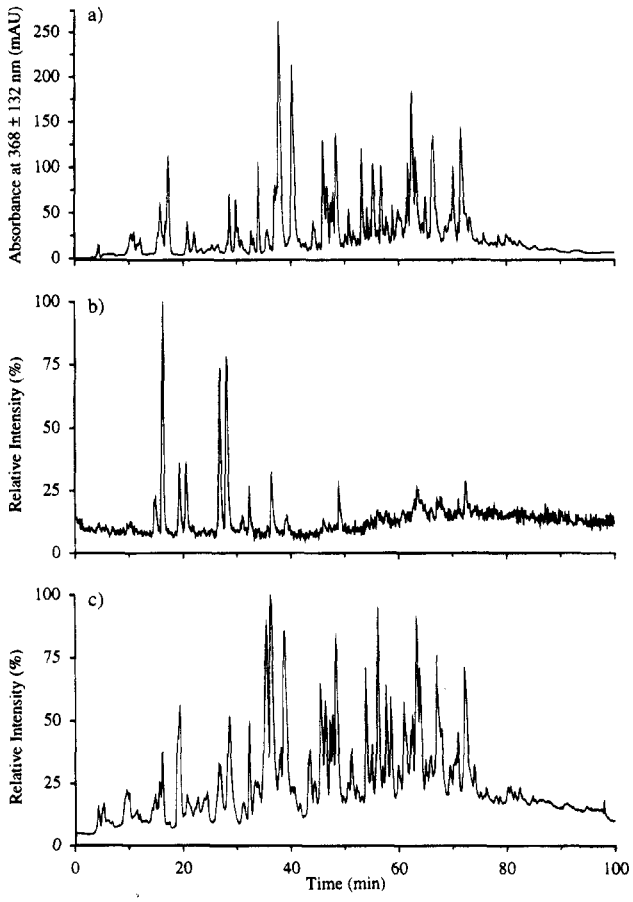

Figure 1. LC analyses of a carbon black extract, using the ternary gradient B (see Experimental Section). (a) UV detection, 236–500 nm; (b) LC/MS total ion chromatogram obtained using the PB interface with EI; (c) LC/MS total ion chromatogram obtained using the HPN interface with APCI. Mass spectrometer scanning parameters are described in the Experimental Section.

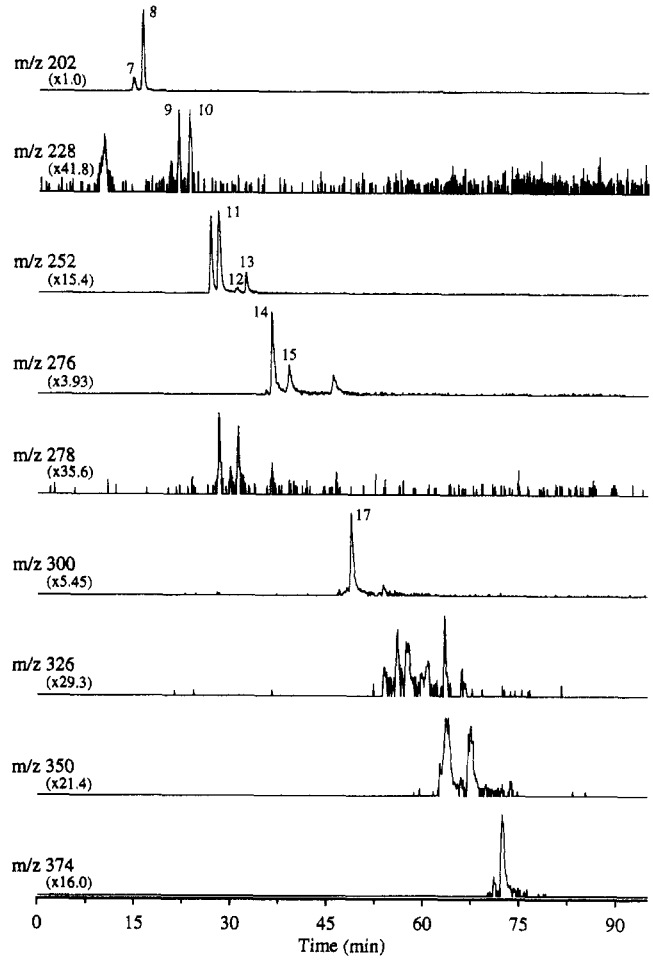

Figure 2. Reconstructed ion chromatograms for $M^{\bullet+}$ ions of PACs, from the same LC/MS analysis of the carbon black extract using the PB interface which was reported as a TIC in Figure 1b. Peak annotations correspond to numbering of analytes in Table 1.

interface. Another problem for the MB interface at lower *m/z* values is the background signal derived from the polyimide belt. The most intense peaks in this background, at *m/z* values of 113, 149, and 167, are readily removed by background subtraction and do not interfere with analyses of common PACs.

The performance of the PB and HPN interfaces in analysis of the same carbon black extract was evaluated using the aqueous acetonitrile/dichloromethane gradient B described in the Experimental Section. Figure 1a presents the LC/UV chromatogram obtained using a diode array detector with a broad bandwidth setting (368 ± 132 nm) detect all PACs. Figure 1b,c shows the TICs resulting from the LC/MS analyses using the PB and HPN interfaces, respectively. Even at this level of comparison, the HPN interface appears to provide superior performance in that the TIC obtained using this interface (Figure 1c) closely matches the LC/UV chromatogram (Figure 1a), whereas the TIC from the PB interface (Figure 1b) provides good response only for the middle-range PACs. The nature of this disappointing performance of the PB interface is made more clear in Figure 2, which shows selected RICs from this LC/MS analysis. No useful data were obtained below *m/z* 200 or above *m/z* 374. The poor performance of the PB interface for the more volatile PACs resembles that of the MB interface, while the difference in performance of these two interfaces for the larger PACs is probably due to the low volatility of these compounds. Thus, samples forming a thin layer on a moving belt are more readily volatilized as a consequence of the benefits of flash heating (by the nose heater in this case), as described by Beuhler et al.[19] and by Daves.[20] The PB interface, on the other hand, requires that small particles of analyte in the beam must strike the back surface of the ion source and be heated quickly enough to vaporize before the next-eluting analyte physically covers the first. Attempts to improve the heat transfer rate, by raising the PB ion source temperature to 300 from 250 °C, resulted in only modest gains in sensitivity.

However, in those cases for which a good response was obtained, the EI mass spectra obtained using the PB interface (e.g., Figure 3a,b) showed much less low-mass background (*m/z* < 150) than did the corresponding spectra obtained using the MB interface.[18] These relatively simple spectra can be readily matched with those contained in mass spectral libraries, although this is of questionable benefit for positive confirmations because most sets of PAC isomers produce identical EI mass spectra.[5] In addition, most alkylated PACs undergo fragmentation under EI conditions. PACs with longer alkyl chains ($>C_3$) usually produce low-intensity molecular ions. In such cases, the EI fragmentation pattern will indicate that the compound is alkylated, but the molecular weight may not be easily determined.

Positive ion APCI spectra of PACs are dominated by singly ionized molecules with no fragmentation and have a high

(19) Beuhler, R. J.; Flanigan, E.; Greene, L. J.; Friedman, L. *J. Am. Chem. Soc.* **1974**, *96*, 3990–3999.
(20) Daves, G. D., Jr. *Acc. Chem. Res.* **1979**, *12*, 359–365.

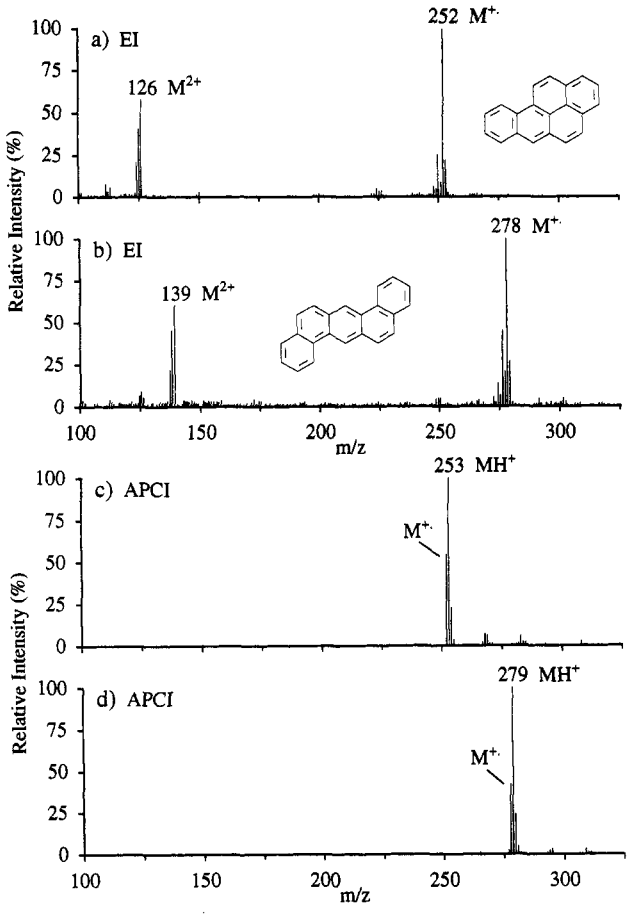

Figure 3. Background subtracted EI (a, b, PB interface) and APCI (c, d, HPN interface) mass spectra of benzo[*a*]pyrene (a, c) and *dibenz[a,h]anthracene* (b, d). Mass spectrometer conditions are described in the Experimental Section.

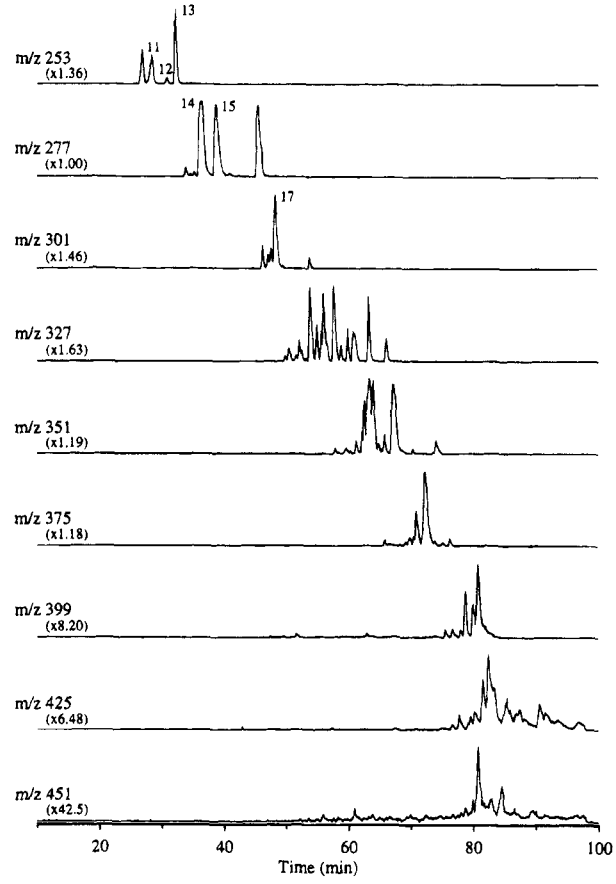

Figure 4. Reconstructed ion chromatograms, from the LC/MS analysis of the carbon black extract using the HPN interface reported as a TIC in Figure 1c. The *m/z* values labeling the RICs are those for the MH^+ ions, since these dominate the $M^{\cdot+}$ ions for these larger PACs. Peak annotations correspond to numbering of analytes in Table 1.

background at $m/z < 250$, which is, however, of constant or slowly varying intensity and can readily be accounted for by background subtraction. The details of the spectra depend upon the composition of the atmosphere in the ion source. The competing ionization mechanisms[21] are proton transfer from protonated water clusters and electron transfer to species such as $N_2^{\cdot+}$, $O_2^{\cdot+}$, and possibly NO^+, to form MH^+ and $M^{\cdot+}$ ions, respectively (e.g., Figure 3c,d). The relative importance of the two mechanisms varies with the partial pressure of water vapor within the plasma created by the corona discharge and can be controlled by doping the ion source with water or benzene vapor, as demonstrated[22] for PAC analysis by supercritical fluid chromatography using APCI mass spectrometry. In general, ionization by protonation was observed to increasingly dominate the electron transfer mechanism as the size of the PACs increased. These trends were confirmed in the LC/MS work reported here, but since ion source doping did not appear to offer any significant advantage, it was not used further in the present work. Some preliminary experiments[18] on negative ion APCI of PAC standards yielded complex mass spectra (not shown) dominated by $(M + 15)^-$ and $(M + 31)^-$ ions, with other higher mass species formed by ion–molecule reactions with the oxide and superoxide anions, which are the main negatively charged constituents of the APCI plasma.[21] These spectra were not very reproducible, in accord with general conclusions about negative ion chemical ionization in the presence of water and oxygen,[23] and this approach was not pursued further.

The performance of the HPN interface in LC/MS analysis of the higher molecular weight PACs in carbon black is illustrated in Figure 4 (the performance of this interface for the smaller PACs is described in the following section). Excellent signal-to-noise ratios are observed for all components observable in the LC/UV chromatogram out to 100 min retention time (Figure 1a). That Figure 4 does not represent an upper limit to the capabilities of the HPN interface, in analysis of large PACs, is suggested by the results of a similar analysis of carbon clusters extracted from a commercial fullerene soot.[24] Compounds up to C_{108} (MW 1296) were clearly detected,[24] and, while fullerenes are not expected to behave in a fashion entirely identical to that of PACs, this evidence suggests that the upper mass limit evident in Figure 4 reflects the composition of the carbon black extract rather than limitations of the HPN interface. This conclusion is also supported by a comparison of Figure 1 parts a and c, which suggests that all components of the carbon black extract which responded to the UV detector were also detected by the mass spectrometer when the HPN interface was used.

(21) French, J. B.; Davidson, W. R.; Reid, N. M.; Buckley, J. A. In *Tandem Mass Spectrometry*; McLafferty, F. W., Ed.; Wiley-Interscience: New York, NY, 1983; pp 353–370.

(22) Anacleto, J. F.; Ramaley, L.; Boyd, R. K.; Pleasance, S.; Quilliam, M. A.; Sim, P. G.; Benoit, F. M. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 149–155.

(23) Stöckl, D.; Budzikiewicz, H. *Org. Mass Spectrom.* **1982**, *17*, 470–474.

(24) Anacleto, J. F.; Boyd, R. K.; Quilliam, M. A. *J. High Resolut. Chromatogr.* **1993**, *16*, 85–89.

It is important to understand the physicochemical origins of this remarkable performance of a device in which the gas temperature reaches no more than 120 °C or so. It seems likely that the key phenomenon is the well-known increase of vapor pressure P over the bulk value P_0 as the sample size is decreased below micrometer dimensions. The quantitative expression of this phenomenon is given by the Kelvin equation:[25-27]

$$\ln(P/P_0) = 2M\gamma/rdRT \quad (1)$$

where M is the molar mass, γ the surface energy per unit area, r the droplet radius, and d the density. For liquids, the effect is not appreciable until r falls below about 10^{-6} cm. However, if the initial nebulization of the LC eluate produces droplets of micrometer dimensions (10^{-4} cm), subsequent evaporation of solvent will leave dry particles of radius no larger than 10^{-6} cm for initial concentrations typical of LC/MS experiments. Moreover, the surface energies of solids are appreciably larger than those of liquids, so the ratio P/P_0 can be considerably greater than unity. Although the Kelvin equation deals with an equilibrium situation, while evaporation *rates* are likely important in the HPN interface, it seems likely that the same parameters controlling the thermodynamics will also control the rate. This explanation can account, at least qualitatively, for the ability of the HPN interface to facilitate acquisition of APCI mass spectra of (sometimes labile) compounds which do not vaporize from the bulk solid.[28]

The present comparison of the three LC/MS interfaces for qualitative profiling of PACs of higher molecular weights has confirmed the utility of the MB interface in this regard, although with notable restrictions on compatible LC mobile phases. The PB interface has no such mobile phase restrictions and provides a better MS background, but it provides very poor response for PACs larger than about 380 Da and less than 200 Da. The HPN interface provided the best performance with respect to both LC compatibility and mass spectrometric response to the full range of PACs present in the carbon black extract. The APCI mass spectra provided by the HPN interface contain only ions derived from intact molecules, while the EI spectra from the MB and PB interfaces contain more information and are suitable for comparisons with library spectra. However, as discussed above, this advantage is of limited practical importance for PAC analysis.

Quantitative Analysis: Standard Calibrations. In previous work from this laboratory, the MB interface was used for the quantitative analysis by LC/MS of PACs in marine sediments.[4,5] For most of the target PACs (300 Da or less), the precision obtained using the MB interface was comparable to that obtained by GC/MS for both external calibration and internal standardization techniques. However, the precision for naphthalene using the MB interface was ~50% relative standard deviation,[4] thus clearly illustrating the difficulty in analyzing volatile compounds when using this interface.

In view of both the previous documentation[4,5] of LC/MS quantification of PACs using the MB interface and also of the mobile phase compatibility limitations of this interface, which in turn limit the isomer separation selectivity (which is one of the major advantages of LC/MS for PAC analysis), only a few confirmatory experiments were conducted in the present work. For example, the performance for coronene (m/z 300) using single ion monitoring (SIM) was excellent, with respect to both instrumental limits of quantification (25 pg) and linear range (2.5 orders of magnitude demonstrated, but probably larger), and was typical of most of the larger PACs studied. The performance for carbazole (dibenzopyrrole), on the other hand, was much worse, with a SIM limit of quantification of 2 ng and a linear range up to about 300 ng with a marked fall-off from linearity above this value. The much lower sensitivity and deviation from linearity at higher sample loadings probably arise from less efficient desorption of the more polar carbazole molecules from the surface of the belt.

The use of the PB interface for quantitative analyses has been seriously hampered because of the nonlinear calibration curves it often produces. Several groups have reported quadratic responses, with slopes increasing with increasing concentration, for a number of analytes.[29-31] It has been suggested[30] that this nonlinear behavior is related to the amount of analyte in a particle and its transmission through the interface. Analyte transport efficiencies are generally less than 10% for most compounds in the particle beam interface.[32] Losses occur primarily in the momentum separator because of particle sedimentation, turbulence, and misalignment of nozzles and skimmer cones.[9] Sedimentation and turbulence losses are directly related to the size of analyte particles, which in turn is a function of analyte concentration[9] and the diameter of the nebulizer tip.[32] Thus, the nonlinear curves are a result of small particles, produced at low analyte concentrations, which are more prone to turbulent losses. More efficient mass transport occurs at higher concentrations as the particle size increases.

The calibration curves obtained for two PAC standards, shown in Figure 5a,b, can be fitted to quadratic equations. Recent reports[33,34] have claimed that, with the exception of benz[*a*]anthracene and chrysene, several PAC standards exhibited linear response behavior over the range of 20–1000 ng. The expansion of the low-mass regions of the two curves (insets, Figure 5a,b) clearly shows that the curve for benzo[*a*]pyrene is nonlinear throughout the entire concentration range, although that for dibenz[*a*,*h*]anthracene reveals acceptably linear behavior (dashed line) below 300 ng injected on-column. Above this level, the calibration curve exhibits the convex downward appearance characteristic of the PB interface. Although nonlinear response does not preclude the use of an instrumental method in quantitative analysis, it does increase the calibration requirements and necessitates interpolation from multipoint calibration curves. As an alternative in the case of the PB interface, the linearization of calibration curves through the use of the "carrier effect" (i.e.,, use of mobile phase additives) has been suggested.[35] Subsequent

(25) Thomson, W. *Phil. Mag.* **1871**, *42*, 448.
(26) Moore, W. J. *Physical Chemistry*, 4th Ed; Prentice Hall: Englewood Cliffs, NJ, 1972; pp 481–482.
(27) Wilemski, G. *J. Chem. Phys.* **1995**, *103*, 1119.
(28) Burlingame, A. L.; Boyd, R. K.; Gaskell, S. J. *Anal. Chem.* **1994**, *66*, 634R–683R.
(29) Brown, F. R.; Draper, W. M. *Biol. Mass Spectrom.* **1991**, *20*, 515–521.
(30) Ho, J. S.; Behymer, T. D.; Budde, W. L.; Bellar, T. A. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 662–671.
(31) Doerge, D. R.; Burger, M. W.; Bajic, S. *Anal. Chem.* **1992**, *64*, 1212–1216.
(32) Browner, R. F.; Winkler, P. C.; Perkins, D. D.; Abbey, L. E. *Microchem. J.* **1986**, *34*, 15–24.
(33) Roby, M. R.; Pace, C. M.; Betowski, L. D.; Marsden, P. J. *Proceedings of the 38th ASMS Conference on Mass Spectrometrty and Allied Topics*; Tucson, AZ, June 3–8, 1990; pp 625–626.
(34) Pace, C. M.; Betowski, L. D. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 597.
(35) Bellar, T. A.; Behymer, T. D.; Budde, W. L. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 92–98.

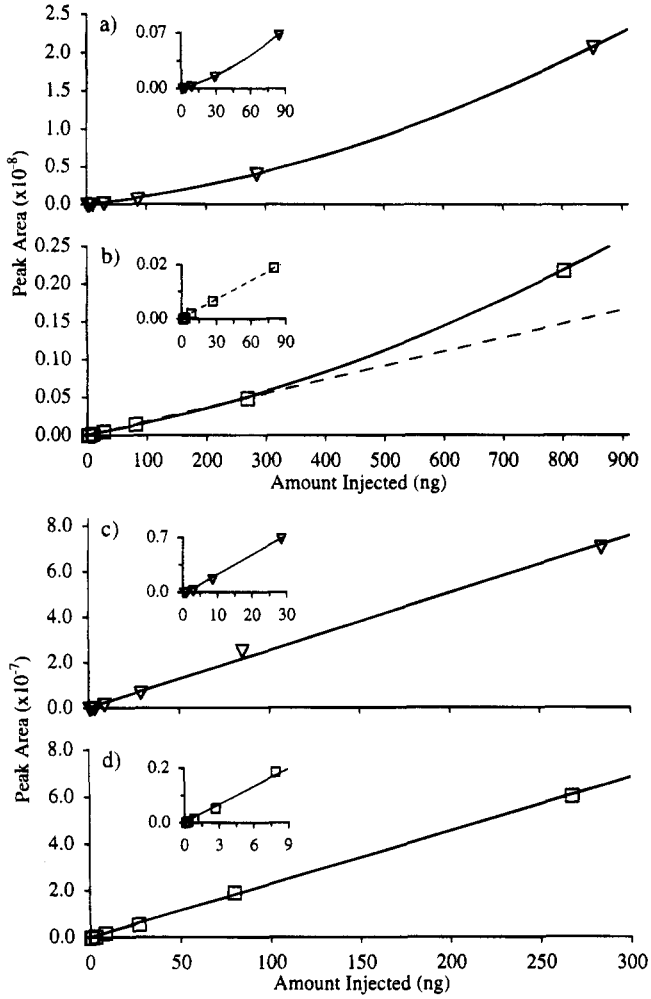

Figure 5. LC/MS calibration curves obtained for benzo[*a*]pyrene (a, c) and dibenz[*a,h*]anthracene (b, d) using the particle beam (a, b) and heated pneumatic nebulizer (c, d) interfaces.

measurements, however, indicated that this technique is limited to very few compounds and that nonlinear behavior was still prevalent.[30] The presence of unexpected coeluting substances in real-world samples can also cause calibration errors and analytical bias. The detector signal given by target analytes has been shown to increase with coeluting matrix constituents.[29] Presumably, particle size discrimination in the momentum separator, with its influence on transport efficiency, is also the basis of this phenomenon.

Instrumental detection limits for the PB interface, determined on the basis of the smallest sample size which yielded a signal-to-noise ratio of at least 3:1, were 3–4 ng injected on-column for benzo[*a*]pyrene and dibenz[*a,h*]anthracene. Previous studies[33,34] of PAC standards using a PB interface also reported detection limits in the low nanogram range. These limits are by no means universal for all PACs; however, the more volatile low molecular weight PACs are more susceptible to losses in the PB interface, thus giving rise to much higher limits of detection and of quantification. This point will be further illustrated below.

The calibration curves obtained for the same two PAH standards using the HPN interface with APCI by monitoring MH^+ ions, are shown in Figure 5c,d. The insets show that the curves are linear down to the lowest concentrations analyzed. Such curves are typical of all the PACs examined thus far using the HPN interface. The dynamic ranges found here are similar to those commonly reported in the literature,[36,37] i.e., at most three decades (10^3). There are several possible explanations for this narrow linear dynamic range. The most likely involves a depletion of APCI reagent ions when high levels of analyte are present, i.e., saturation of the APCI ionizing power. Other possible explanations involve saturation of the pulse-counting ion detection system incorporated in the instrument used for the HPN work.

Instrumental detection limits for the HPN interface, determined on the basis of the smallest amount of analyte which yielded a signal-to-noise ratio of at least 3:1, were 275 and 85 pg for benzo[*a*]pyrene and dibenz[*a,h*]anthracene, respectively. These detection limits are considerably lower than those of the particle beam interface (between 1 and 4 ng). The difference of a factor of 3 between the values obtained for these two compounds is difficult to explain in terms of gaseous ion properties, since the two ionization energies are very close[38] (7.12 ± 0.01 and 7.38 ± 0.04 eV, respectively) and, using the correlations of Meot-Ner,[39] the corresponding proton affinities are estimated to be 900 and 870 kJ mol^{-1}. These values are sufficiently close that it seems reasonable that the ratios of intensities of $M^{\bullet+}$ and MH^+ ions should be about the same for these two PAHs, as was observed in Figure 3c,d. It is also difficult to account for this difference in sensitivities in terms of information on the vapor pressures of the two solid PAHs.[40-42]

Quantitative Analysis of the NIST Certified Reference Material SRM 1597. The NIST reference material SRM 1597 is a natural complex mixture of PACs isolated from coal tar, certified for concentrations of 12 PAHs ranging from naphthalene (128 Da) to benzo[*ghi*]perylene (276 Da). The preparation and certification of this reference material have been described by Wise et al.[43] Information values for a further 18 PACs (including some PAHs) are also supplied in the NIST certification document,[44] and more recently an information value for the concentration of dibenz[*a,h*]anthracene was published.[45] This well-characterized material was chosen as a suitable test case for LC/MS analyses.

The present experiments, designed to obtain calibration curves for quantification by external standardization exemplified by Figure 5, indicated that use of internal standards would be mandatory for the PB interface. Particle beam MS has also been shown to be susceptible to tremendous fluctuations in absolute response during intra- and interday runs.[46] For example, instrument response factors for chlorinated phenoxy acid standards varied as much as 2-fold over a 24 h period.[47] This presents another significant obstacle when using an external calibration.

(36) Gilbert, J. D.; Hand, E. L.; Yuan, A. S.; Olah, T. V.; Covey, T. R. *Biol. Mass Spectrom.* **1992**, *21*, 63–68.
(37) Fouda, H.; Nocerini, M.; Schneider, R.; Gedutis, C. *J. Am. Soc. Mass Spectrom.* **1991**, *2*, 164–167.
(38) Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. Gas Phase Ion and Neutral Thermochemistry. *J. Phys. Chem. Ref. Data* **1988**, *17*, Suppl. 1.
(39) Meot-Ner, M. *J. Phys. Chem.* **1980**, *84*, 2716–2723.
(40) Wakayama, N.; Inokuchi, H. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2267–2271.
(41) Murray, J. J.; Pottie, R. F.; Pupp, C. *Can. J. Chem.* **1974**, *52*, 557–563.
(42) White, C. M. *J. Chem. Eng. Data* **1986**, *31*, 198–203.
(43) Wise, S. A.; Benner, B. A.; Byrd, G. D.; Chesler, S. N.; Rebbert, R. E.; Schantz, M. M. *Anal. Chem.* **1988**, *60*, 887–894.
(44) U.S. National Institute of Standards and Technology, SRM 1597 Certificate of Analysis, 1987.
(45) Wise, S. A.; Deissler, A.; Sander, L. C. *Polycyclic Aromat. Compd.* **1993**, *3*, 169–184.
(46) Brown, M. A.; Stephens, R. D.; Kim, I. S. *Trends Anal. Chem.* **1991**, *10*, 330–336.

The obvious solution is through use of internal standards, especially isotopically labeled internal standards, which exhibit chemical behavior almost identical to that of the native substances but are readily differentiated by their mass spectra. If internal standards are chromatographically resolved from the target analytes (which is true for perdeuterated PACs), different matrix effects can still cause calibration errors through coelution of a matrix constituent with either the internal standard or the analyte.[29] The only reliable means to guarantee no selective enhancement of either the internal standard or the analyte, when using the PB interface, is through the use of coeluting isotopically labeled standards, e.g., ^{13}C-labeled standards which are, however, of limited availability and are expensive.

Experiments were conducted to determine whether perdeuterated PAC internal standards (chromatographically resolved from their native perprotonated counterparts) can be used to quantify PACs in a complex sample. A mixture of perdeuterated PAH standards (DPAC-1) was spiked at four different levels into both a certified NIST PAH standard mixture (SRM 1647) and a complex mixture of PACs from coal tar (SRM 1597). A total of 24 *m/z* values were monitored (17 PAHs including several sets of isomers, and appropriate perdeuterated PAHs) using LC/MS with the PB interface. A typical analysis of a SRM 1647/DPAC-1 mixture (10:1 volume ratio) is shown as Figure 6. Only seven of the 17 target PAHs were detected (Figure 6, second acquisition period) and subsequently quantified. The lower molecular weight PAHs (*m/z* 178 and lower), which were present in much higher concentrations than the other components (see Table 1), were among the PAHs that were not detected (Figure 6, first acquisition period). These compounds were too volatile to be efficiently transported through the PB interface. The higher molecular weight PAHs (*m/z* 276 and higher), which were present in concentrations similar to those of the PAHs that were successfully quantified (see Table 1), were also not detected (Figure 6, third acquisition period). These compounds are apparently insufficiently volatile to vaporize efficiently in the hot ion source. These discrimination effects, which were also noted in the qualitative analysis of the carbon black extract (Figure 2), represent a severe disadvantage of the PB interface.

Analysis of the same SRM 1647/DPAC-1 mixture (10:1 volume ratio), by LC/MS using the HPN interface with APCI, is shown in Figure 7. A total of 48 *m/z* values were monitored (both $M^{\bullet+}$ and MH^+ ions of 17 PAHs, including several sets of isomers, and their perdeuterated counterparts). In contrast to the results obtained using the PB interface, all 16 of the target PAHs (and also coronene) were easily detected with excellent signal-to-noise ratios and were subsequently quantified. The LC peak observed in the SIM trace for *m/z* 302 (Figure 7) probably represents interference from the ^{13}C isotopomers of the coronene ions.

Calibration curves were generated using the data from the four mixed SRM 1647/DPAC-1 solutions (raw data for only one mixture are shown in Figures 6 and 7). Plots of the protonated to deuterated PAH peak area ratios versus the known molar ratios (measured as SRM 1647/DPAC-1 weight ratios) for benzo[*b*]-fluoranthene (B*b*F) are shown in Figure 8 for the PB and HPN interfaces. For all those PAHs that were detected with reasonable sensitivity using the PB interface (Figure 6), the calibration plots resembled that shown in Figure 8, in that they could be described

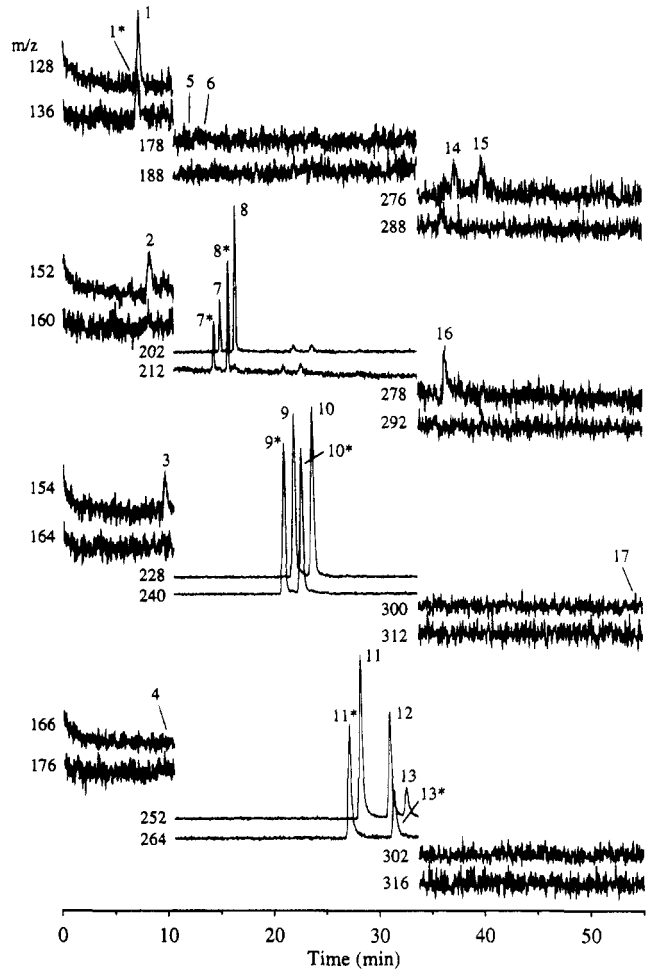

Figure 6. Analysis of a SRM 1647/DPAC-1 mixture (10:1 v/v) using particle beam LC/MS and ternary gradient C (see Experimental Section). A total of 24 *m/z* values were monitored in three acquisition periods: period 1 (0–9.8 min), *m/z* 128, 136, 152, 160, 154, 164, 166, 176; period 2 (9.8–33.5 min), *m/z* 178, 188, 202, 212, 228, 240, 252, 264; period 3 (33.5–60 min), *m/z* 276, 288, 278, 292, 300, 312, 302, 316. These *m/z* values represent $M^{\bullet+}$ ions of PAHs and their perdeuterated versions (peaks marked with an asterisk represent the perdeuterated PAH internal standards). See Table 1 for compound identities. The SIM chromatograms are labeled with their respective *m/z* values and are shown offset from one another vertically for the sake of clarity of presentation.

either as linear with non-zero intercepts or as quadratic curves including the origin. In either case, the nonideality can be interpreted in terms of lower transmission efficiencies for the native analyte than for its perdeuterated counterpart. Negative *y*-intercepts for forced linear fits, such as that observed in Figure 8 for the PB interface, are usually interpreted in terms of irreversible losses of analyte somewhere in the analytical train.[48] In this instance, analyte losses are known to occur in the PB interface, but the dependence of these losses on total sample loading makes difficult any detailed interpretation.

The calibration curve obtained for B*b*F using the HPN LC/MS interface with APCI in the analyses of the NIST 1647/DPAC-1 mixtures is also shown in Figure 8. The linear regression curves for this, and for all the other target PAHs,[7] did include the origin to within experimental error. Regression coefficients were mostly ≥ 0.99, although a few were not as high. For the best precision

(47) Kim, I. S.; Sasinos, F. I.; Stephens, R. D.; Wang, J.; Brown, M. A. *Anal. Chem.* **1991**, *63*, 819–823.

(48) Boyd, R. K. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 257–271.

Table 1. Results of LC/MS Analyses of a Coal Tar Reference Material (NIST SRM 1597) Compared with Certified and Information Values[a]

			particle beam interface			heated pneumatic nebulizer		
no.	compound	certfd concn	LC/MS concn	RSD, % (n = 3)	reltv error, %	LC/MS concn	RSD, % (n = 3)	reltv error, %
1	naphthalene ($C_{10}H_8$)	1160 ± 50				1149	2.9	−1.0
2	acenaphthylene ($C_{12}H_8$)	250[b]				247	3.6	−1.1[b]
3	acenaphthene ($C_{12}H_{10}$)					9.2	1.0	
4	fluorene ($C_{13}H_{10}$)	140[b]				161	4.6	−15[b]
5	phenanthrene ($C_{14}H_{10}$)	462 ± 3				461	3.2	−0.1
6	anthracene ($C_{14}H_{10}$)	101 ± 2				107	5.4	6.1
7	fluoranthene ($C_{16}H_{10}$)	322 ± 4	338	13	4.9	351	11	9.0
8	pyrene ($C_{16}H_{10}$)	235 ± 2	238	11	1.2	241	5.0	2.6
9	benz[*a*]anthracene ($C_{18}H_{12}$)	98.6 ± 3.6	97.2	2.4	−1.4	105	4.6	6.8
10	chrysene ($C_{18}H_{12}$)	71.7 ± 1.0	59.6	1.5	−17	70.4	7.5	−1.8
11	benzo[*b*]fluoranthene ($C_{20}H_{12}$)	66[b]	64	2.1	−3.0[b]	63	7.9	−4.5[b]
12	benzo[*k*]fluoranthene[d] ($C_{20}H_{12}$)	43[b]	41	5.5	−4.7[b]	41	8.0	−4.7[b]
13	benzo[*a*]pyrene ($C_{20}H_{12}$)	95.8 ± 5.8	94.7	4.7	−1.2	84.8	1.1	−11
14	benzo[*ghi*]perylene ($C_{22}H_{14}$)	53.7 ± 7.6				57.0	3.3	6.2
15	indeno[*1,2,3-cd*]pyrene[e] ($C_{22}H_{12}$)	60.2 ± 4.4				60	25	−0.4
16	dibenz[*a,h*]anthracene ($C_{22}H_{14}$)	6.8[c]				7.4	6.6	8.8[c]
17	coronene ($C_{24}H_{12}$)	11[b]				10.7	1.2	−2.7[b]

[a] Concentrations in μg/g. Internal standards were the perdeuterated compounds (DPAC-1[7]) except where noted. [b] Values not certified by NIST; information values reported by NIST.[44] [c] Value not certified by NIST; information value obtained by HPLC by NIST.[45] [d] Perdeuteriobenzo[*b*]fluoranthene was used as internal standard. [e] Perdeuteriobenzo[*ghi*]perylene was used as internal standard.

and accuracy in quantitative analyses of this kind, multipoint calibrations and 1:1 concentration ratios (target analyte/internal standard) should be used, but the highly linear calibration curves obtained using the HPN interface reduce the stringency of this requirement.

Calibration curves such as those illustrated in Figure 8 were used to determine PAH concentrations in the NIST SRM 1597 coal tar extract, spiked with the DPAC-1 standard solution. Most of the values obtained using the HPN interface were reported previously,[7] though with less experimental detail, and are included here for comparison with the results obtained using the PB interface. Both sets of values, together with the NIST certified concentrations[44] or information values, [44,45] are listed in Table 1 for those 15 compounds whose perdeuterated analogs are included in the DPAC-1 solution or for which closely eluting perdeuterated compounds in DPAC-1 can reasonably be used as internal standards. In addition, the concentration obtained for acenaphthene (not covered by the NIST work[44,45]) is included for information.

Except for fluoranthene and chrysene, the accuracies of the concentration values obtained using the PB interface are within acceptable limits. The low concentration obtained for chrysene appears to be a result of matrix components coeluting with its perdeuterated internal standard. A full-scan analysis of the coal tar extract revealed the presence of components at *m/z* 216 (either a benzofluorene or a methylpyrene isomer) and 226 (probably cyclopenta[*cd*]pyrene) whose retention times matched those of chrysene and its perdeuterated counterpart. The chromatographic peaks of the other PAHs which were quantified were also thoroughly examined, and except for perdeuteriobenzo[*b*]fluoranthene, they all appeared free from coeluting components. The perdeuteriobenzo[*b*]fluoranthene peak was not completely resolved from another PAH of molecular mass 252 Da (probably perylene), but not to an extent sufficient to significantly affect the result listed in Table 1. In the case of fluoranthene, another PAH isomer of molecular mass 202 Da appeared to coelute, as indicated by the observation that the front baseline of the LC peak was slightly but significantly broadened. The NIST SRM 1597 certificate of analysis[44] does indicate the presence of a third isomer (acephenanthrylene) at a concentration (uncertified) of ~60 μg/g. An assumption that the present experiments measured the sum of concentrations of fluoranthene plus this isomer, together with the probable PB transmission enhancement from the matrix effect, could account for the high fluoranthene concentration reported in Table 1.

The deviations of the concentrations measured by LC/MS using the HPN interface, from the certified values, were <7.0%, with the exception of those for fluorene, fluoranthene, and benzo[*a*]pyrene, as discussed previously.[7] The partial coelution of another PAH isomer of molecular mass 202 Da (probably[44] acephenanthrylene) with fluoranthene, and of an isomer of fluorene (*m/z* 166) in the coal tar solution, is believed to be the reason for the high values determined in the present work (Table 1). However, the LC peaks for both benzo[*a*]pyrene and its perdeuterated analog were completely resolved from any interferences. At this time, no explanation for this low measured concentration (Table 1) can be given.

CONCLUSIONS

The present findings concerning the relative merits of the MB, PB, and HPN interfaces for LC/MS analyses of PACs appear to reflect some of the more general impressions of these three devices. The MB interface is mechanically awkward, does not permit routine use of gradient elution with aqueous mobile phases due to related variations in pumping and heating requirements in the solvent removal stages, shows poor transmission efficiencies for the more volatile PACs, and provides a significant mass spectrometric background. On the other hand, the MB interface provides good quality EI spectra for high molecular weight PACs not amenable to GC/MS analysis and can also readily be used with chemical ionization, though this was not demonstrated here. Previous work from this laboratory[4,5] has demonstrated that, with some effort, acceptable quantification of the less volatile PACs in complex mixtures can be achieved using the MB interface.

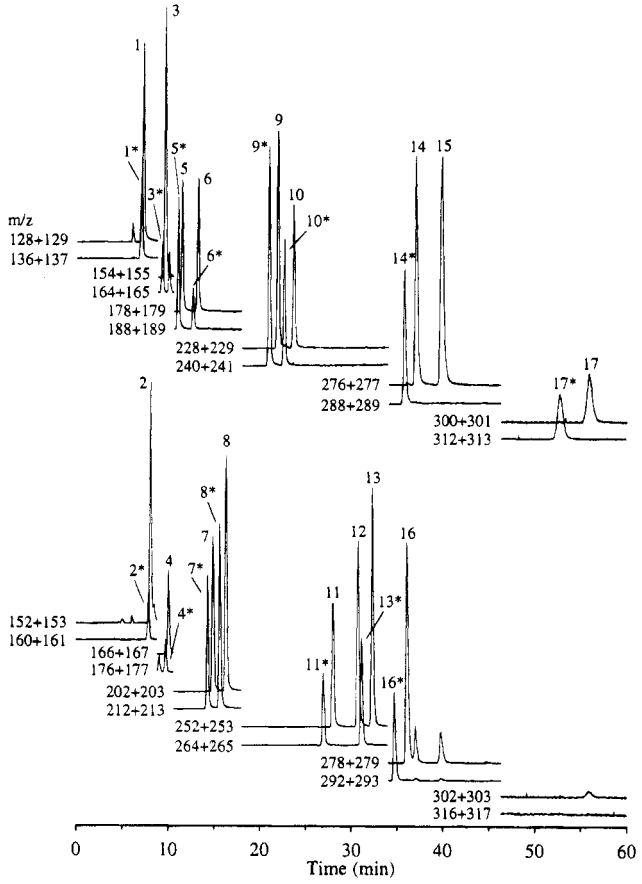

Figure 7. Analysis of a SRM 1647/DPAC-1 mixture (10:1 v/v) using the HPN LC/MS interface with APCI and ternary gradient C (see Experimental Section). A total of 48 ions (both $M^{\bullet+}$ and MH^+ ions of target PAHs and their perdeuterated versions) were monitored in six acquisition periods: period 1 (0–8.8 min), *m/z* 128 + 129, 136 + 137, 152 + 153, 160 + 161; period 2 (8.8–10.5 min), *m/z* 154 + 155, 164 + 165, 166 + 167, 176 + 177; period 3 (10.5–18 min), *m/z* 178 + 179, 188 + 189, 202 + 203, 212 + 213; period 4 (18–33.4 min), *m/z* 228 + 229, 240 + 241, 252 + 253, 264 + 265; period 5 (33.4–46 min), *m/z* 276 + 277, 288 + 289, 278 + 279, 292 + 293; period 6 (46–60 min), *m/z* 300 + 301, 312 + 313, 302 + 303, 316 + 317. See Table 1 for compound identities. Peaks marked with an asterisk represent the perdeuterated PAH internal standards. For the sake of clarity of presentation, the SIM chromatograms are shown offset from one another vertically.

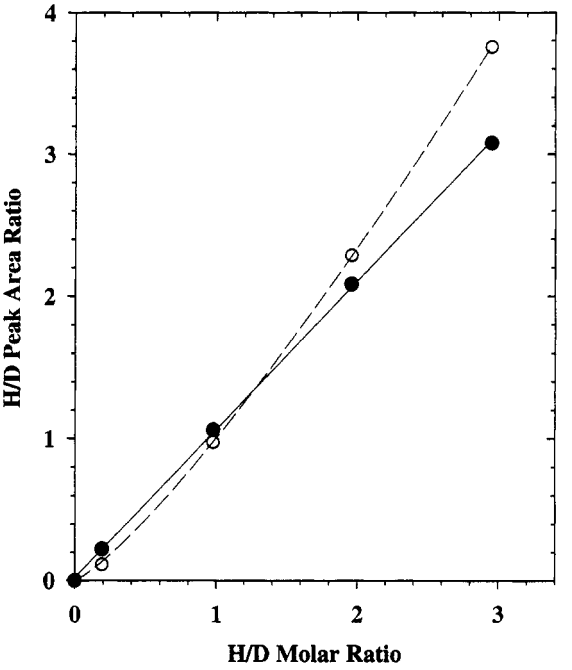

Figure 8. Calibration curves for the analysis of benzo[*b*]fluoranthene (B*b*F) using the PB (○) and HPN (●) LC/MS interfaces and using perdeuterio-B*b*F as an internal standard. Solutions were prepared by mixing varying proportions of SRM 1647 and DPAC-1 standard solutions. Each point represents the mean of triplicate measurements of the ratios of peak areas for the $M^{\bullet+}$ ions of perprotio- and perdeuterio-B*b*F. The dashed curve (PB interface) represents a least-squares fit to an assumed form ($y = ax^b$), with $a = 1.006$, $b = 1.218$, and $r^2 = 0.998$. The full curve (HPN interface) represents a fit to the linear form ($y = cx + d$), where $c = 1.042$, $d = 0.0361$, and $r^2 = 0.990$.

The PB interface is compatible with aqueous mobile phases and provides EI spectra with appreciably less background than does the MB interface. However, the PB interface exhibits poor transmission efficiencies for PACs of both low (<200) and high (>380) molecular weights. The highly nonlinear calibration curves and poor detection limits obtained using the PB interface, together with the marked carrier effects due to coeluting compounds, make the PB interface difficult to use reliably in quantitative PAC analyses, even when perdeuterated PAC internal standards are employed.

The HPN interface with APCI provided the best overall performance in the present work. This LC/MS interface is compatible with a wide range of mobile phase compositions, exhibits excellent transmission efficiencies and detection limits for both low and high molecular weight PACs, and provides excellent linearity of response. The main disadvantages of the HPN interface encountered in the present work were the lack of fragment ions and thus of structural information (though this feature is of limited importance for PACs, and in general, concentration of ion current in ionized molecular species is an advantage for SIM experiments) and the limited dynamic range (about 10^3), although it is possible that the latter may reflect the pulse-counting detection system of the APCI instrument used in the present work. The mass spectrometric background at low *m/z* values, while significant, varies slowly during the LC elution and thus provides a constant baseline for quantification by selected ion monitoring or can be corrected for by background subtraction for full mass spectral acquisition.

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