Anal. Chem. **2001,** *73,* 3691–3700

Characterization of Asphalt Fume Composition under Simulated Road Paving Conditions by GC/MS and Microflow LC/Quadrupole Time-of-Flight MS

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A highly sensitive, selective, and reliable analytical method has been developed and validated for characterization of asphalt fume generated under simulated road paving conditions. A dynamic asphalt fume generation system was modified to provide consistent test atmospheres at simulated asphalt road paving conditions. In the process of fume generation, asphalt was initially preheated in an oven to 170 °C, pumped to a large kettle, which maintained the asphalt temperature between 150 and 170 °C, and then transferred to the generator. The fume was conducted from the generator to an exposure chamber through a heated transfer line. Characterization of the asphalt fume test atmospheres included the following: (1) determination of the consistency of the asphalt aerosol composition within the generation system; (2) quantification of total organic matter of the asphalt fume by electron impact ionization of isotope dilution gas chromatography/mass spectrometry); and (3) identification of individual priority polycyclic aromatic hydrocarbons (PAHs) in asphalt fume by selected ion monitoring. With the developed method, asphalt fumes could be characterized into three fractions: (1) filter collection of a large molecular size fraction over a range of mass-to-charge (m/z) ratios of 173–309; (2) XAD-2 trapping of a medium molecular size fraction over a range of m/z ratios of 121–197; and (3) charcoal trapping of a small molecular size fraction that contained mainly the volatile vapor fraction over a range of m/z ratios of 57–141. Total organic matter of the asphalt fume was quantified over the 5 exposure days. Sixteen specific priority PAHs were monitored and identified. These PAHs were determined at trace levels on the filter fraction. A novel approach, which utilizes collision-induced dissociation of fragmentation pathway leading to a characteristic fragmentation pattern by coupling microflow liquid chromatography to atmospheric pressure chemical ionization of quadrupole time-of-flight mass spectrometry, was used to further clarify the trace amount of key components present in simulated road paving asphalt fumes. These results demonstrate that asphalt fume composition could be characterized and specific priority PAHs could be identified by this method. The major advantages of this method are its highly sensitivity, selectivity, and reliability for chemical hazard characterization in a complex mixture. This method is suitable for support toxicity studies using simulated occupational exposure to asphalt fumes.

Millions of tons of asphalt are produced every year and extensively used in the paving and roofing industries.[1] It is estimated that ~4000 hot-mix asphalt facilities and 7000 paving contractors employ nearly 300 000 workers in the United States.[2] Therefore, a considerable number of workers in those industries are potentially exposed to asphalt fumes. Occupational exposure to asphalt fumes may pose a health risk to workers. The worker exposure routes are mainly through inhalation and skin contamination. It had been reported that European road paving workers can be exposed to 0.1–2 mg/m^3 bitumen fume, which can include 10–200 ng/m^3 benzo[*a*]pyrene.[3] Although the levels of exposure of these workers were low, there is a concern of long-term health effects following chronic exposure.

Asphalt is an extremely complex and variable mixture. It contains aliphatic, polycyclic aromatic hydrocarbons (PAHs), heterocyclic compounds, and some nitrogen-, oxygen-, and sulfur-containing compounds.[4] These persistent organic compounds are very nonpolar and exhibit a high accumulation potential in living systems.[5] The highly lipophilic PAH chemicals constitute an extraordinarily large and diverse class of organic molecules and represent components with a wide range of molecular sizes and structural types. It has been estimated that crude asphalt contains the most widely distributed class of potent carcinogens present

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in the human environment.[6] Moreover, hundreds of different PAHs have numerous isomers, and the number of isomers increases almost exponentially with molecular weight, which makes chemical analysis of these compounds a significant challenge.

Research on asphalt fume exposure has been carried out for many years. A major health concern from asphalt fume exposure is the potential exposure to carcinogens. Twenty epidemiological investigations on the cancer risk in asphalt road pavers and roofers were evaluated.[7] The authors concluded that there were indications of increased risks for lung, stomach, and nonmelanoma skin cancers and for leukemia in roofer populations. The relative risks for the same cancers in road pavers and highway maintenance workers were lower than those in roofers. Laboratory studies on asphalt fume condensates generated from roofing asphalt in the laboratory at 316 °C had shown that asphalt fumes were mutagenic[8] and caused skin tumors when asphalt fume condensates were painted onto mice.[9]

Chemical characterization of asphalt fumes is important to elucidate toxic mechanisms initiated by asphalt fume exposure. It is also vital in assessing the sources of these chemical hazards. PAHs represent a very important group of chemical carcinogens in asphalt. The adverse effects of PAHs are recognized and have been documented in epidemiological studies.[10] It has been estimated that PAHs containing four or more rings were responsible for over 75% of the carcinogenic potential of this class of compounds.[11] Factors such as the temperature and the variable pitch content of the binder can lead to extraordinary variation in composition and toxicity of the fumes.[12] Information regarding exposure to potential carcinogenic compounds from asphalt fumes is still limited. Assessing occupational exposure to asphalt fume is especially problematic because methodologies for evaluating a complex exposure mixture are not well established. Therefore, the major objectives of this study were to develop and validate a reliable analytical method for chemical hazard identification and quantification in this complex mixture and to support the development of an asphalt fume generator, which simulates occupational exposure conditions for inhalation toxicity studies. The research included characterization of the asphalt fume composition of an aerosol designed to simulate road paving conditions and determination of the stability of the fume generation system. On the basis of this study, the longer term goal of our study was to develop strategies through assessment of biomarkers for predicting and monitoring adverse health affects and to try to link key component/chemical classes to specific biological effects in occupational mixed exposure studies.

EXPERIMENTAL SECTION

Materials and Instruments. Reagent dichloromethane (99.9+%) and hexane (capillary GC grade) were purchased from Aldrich (Milwaukee, WI). QTM PAH Mix standard was purchased from Supelo (Bellefonte, PA). Poly(tetrafluoroethylene) (PTFE) filters (37 mm, 0.45-μm pore size), XAD-2 traps [treated with 2-(hydroxymethyl)piperidine], and charcoal absorption tubes were purchased from SKC (Eighty Four, PA). Solid-phase extraction cartridges of Bond Elut CN-U (500 ng/2.8 mL) and SI silica (500 mg/2.8 mL) were purchased from Varian (Harbor City, CA). PTFE tubes (30 mL) and glass tubes (10 mL) were purchased from Fisher Scientific (Pittsburgh, PA). Target DP vials (1.5 mm with 200-μL inserts) were obtained from Alltech Associates, Inc. (Deerfield, IL). Extraction of asphalt fumes from collection media was performed by ultrasonic extraction (FS-220, Ultrasonic power 320 W, Fisher Scientific, Fairlawn, NJ). A BenchMate II Workstation (Solid-Phase Extractor, Zymark, Hopkinton, MA) and syringe filter (25 mm, 0.2-μm pore size) specially designed for HPLC samples (Gelman Sciences, Ann Arbor, MI) were employed to perform the purification and filtration of the sample solutions. Extracts were evaporated under a nitrogen stream using a TurboVap LV evaporator (Zymark). Liquid nitrogen, high-purity helium, and argon were purchased from Butler Gas Products Co. (Mckees Rocks, PA), and used as gas chromatography/mass spectrometry (GC/MS) and quadrupole time-of-flight (Q-TOF) MS carrier gases. Methane (Butler Gas Products Co.) was employed as a reagent for chemical ionization. The GC column used was HP-5 MS, 95% dimethylpolysiloxane, nonpolar, 30-m length, 0.53-mm i.d. (Hewlett-Packard, Wilmington, DE), and the microflow LC column used was Nucleosil C_{18} PAH, 5 μm, 1000-μm i.d. (LC Packings, San Francisco, CA). The test asphalt was used by the paving industry (Hot Performance Grade Asphalt PG 64-22). Asphalt fumes were characterized by GC/MS (Hewlett-Packard) and coupling a microflow LC (LC packing) to APCI of Q-TOF MS (Micromass Inc., Beverly, MA).

Asphalt Fume Generation System. Generation of asphalt fume was conducted in the National Institute for Occupational Safety Health inhalation facility. A dynamic asphalt fume generation system (Heritage Research Group, Indianapolis, IN) was modified to provide test asphalt fumes. The system modifications are shown in Figure 1. A computer control system was designed to improve the system's performance and to simplify its operation. The test asphalt was representative of the type used by the paving industry throughout the midwestern United States and was supplied by the Heritage Research Group. For fume generation, the asphalt was initially preheated in an oven to 170 °C and then pumped to a large bitumen kettle, which maintained the asphalt temperature between 150 and 170 °C. The heated asphalt was then transferred to the generator (inlet temperature of 145−150 °C), and the fume was generated above the asphalt surface as the asphalt flowed along the generator plate. Air passing over the surface of the asphalt mixed with the asphalt fume. The resulting mixture was transferred from the generator to the exposure chamber through a short heated transfer line.

Collection of Asphalt Fume Samples. The primary strategy used in dealing with asphalt aerosol collection consisted of filter collection of particles, followed by appropriate second-stage trapping of the vapor fraction. In comparison of sampling ef-

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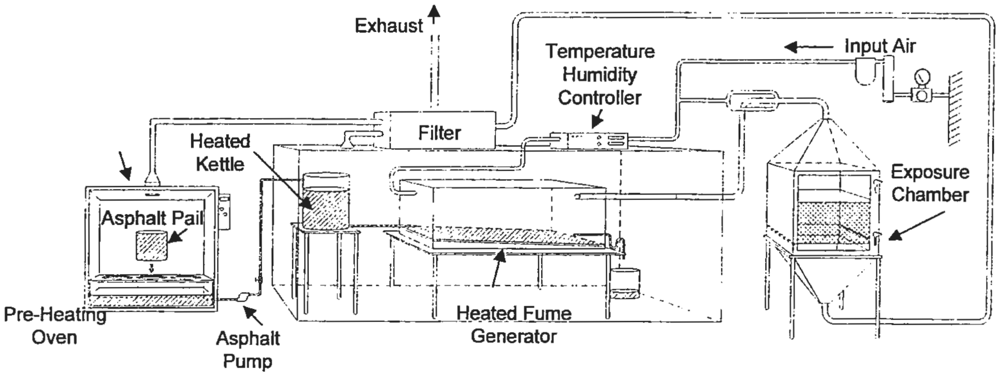

Figure 1. Diagram of the modified asphalt fume generation system.

ficiency, two sampling trains were tested. The first train employed sampling through a PTFE filter (0.45-μm pore size), followed by a −190 °C cold trap to collect the volatile fraction. Another sampling train consisted of a PTFE filter, an XAD-2 trap [treated with 2-(hydroxymethyl)piperidine], and a charcoal trap in series at a flow rate of 1.0 L/min. Asphalt fume samples were collected and analyzed at various points throughout the generation system including the asphalt oven, kettle, generator, and chamber to monitor the uniformity of the asphalt fume aerosol distribution within the generator and the exposure chamber.

Preparation of Samples. A few processing steps were required and performed to manipulate the asphalt fume samples into a form ready for analysis. In addition, the proper concentration range of samples necessary to introduce samples into the instrument was determined. Sample preparation involved desorption, filtration, and preconcentration to determine the total organic matter of asphalt fume. The samples from either the PTFE filter or XAD-2 trap or charcoal absorbants were transferred to separate PTFE tubes, and dichloromethane/hexane was added. Ultrasonic extraction was performed using a FS-220 ultrasonicator (320 W). After desorption of asphalt fume from the collection medium, the extract solution was filtered through into a test tube using a BenchMate II workstation, which was programmed to perform filtration procedures automatically. A PTFE filter specially designed for HPLC samples was used for filtration of the sample solution. Preconcentration was performed by reducing the solvent of sample solution under a nitrogen stream using a TurboVap LV evaporator. Sample extracts were reconstituted with dichloromethane.

Additional cleanup procedures were required for isolation of individual priority polycyclic aromatic hydrocarbons (PAHs) in asphalt fume samples. Two purification stages were performed using solid-phase extraction to remove impurities that could interfere with proper interpretation of the results. The asphalt fume was extracted from the collection medium. The extract was transferred to a smooth-walled test tube. This tube was loaded onto the sample position of a BenchMate II workstation. The first purification stage was performed using a Bond Lut CN-U cartridge. The CN-U cartridge was conditioned with methanol and then with hexane. The sample was eluted through the cartridge and collected. The CN-U cartridge was washed with 0.5 mL of hexane and collected in the same tube as the initial elutes. The second purification stage was performed using a Bond Lut SI silica cartridge. The SPE procedures were performed in a manner similar to those used for the CN-U cartridge. Then, the extracts were filtered with a TPFE filter (25 mm and 0.2-μm pore size) by the BenchMate II workstation. In operation, an editing program performed both SPE and filtration procedures automatically. Preconcentration was performed by evaporating the solvent of the samples under a nitrogen stream using a TurboVap LV evaporator. Procedural blanks of filter, XAD-2, and charcoal were prepared using the entire analytical procedure as well as the same reagents as those used for treating asphalt fume samples.

GC/MS and Microflow LC-Q-TOF MS Detection. The determination of asphalt fume composition was carried out using both positive electron ionization (EI) and chemical ionization (CI) of GC/MS. The high sensitivity of EI was used to quantify the total organic matter of asphalt fume samples. The total ion chromatograms were acquired with 3-min solvent delay for GC/MS. Separation was performed using a temperature program from 50 to 310 °C at an increasing rate of 5 °C/min with an HP-5 MSD capillary column (30-m length, 0.53-mm i.d). Structural information was obtained from the fragmentation pattern of EI. CI was used to characterize asphalt fume fractions by providing molecular ion information from the intact molecule. In considering different applications, both CI and EI techniques were used. To detect individual priority PAHs in asphalt fume at trace levels, a selected ion monitoring (SIM) mass spectrometer was used. In the SIM mode of operation, the detection system was dedicated to monitoring only a few ion currents. Therefore, the sensitivity was tremendously higher than the scanning mode.[13] In addition, a high-performance Q-TOF MS instrumentation was employed to clarify the key PAH components from asphalt fume samples. A mixture of 16 priority standard PAHs was used to calibrate both GC/MS and Q-TOF MS instruments. Analytical procedural blanks for filter, XAD-2, and charcoal samples were employed to check the contamination and were analyzed with each batch of samples.

RESULTS AND DISCUSSION

Analytical Methodologies. Asphalt exposure assessment is complicated by the following: (1) the complex and variable nature of asphalt; (2) the lack of a single chemical substance representative of asphalt fume exposure; and (3) the fact that no single sampling and analytical procedure is capable of covering all components required for assessment. Therefore, the successful

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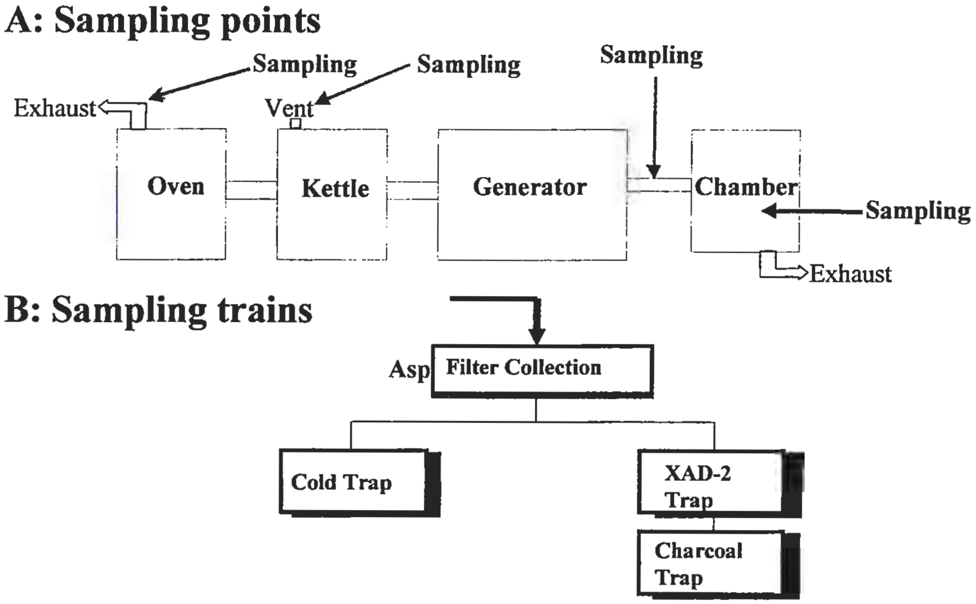

Figure 2. Sampling method: (a) sampling points; (b) sampling trains.

characterization of asphalt fume exposure depends on the proper development of analytical methodologies. In this study, advanced mass spectrometric techniques, including GC/MS and the coupling of microflow LC to a Q-TOF MS, were used to develop a highly sensitive, selective, and reliable analytical method. In addition, various combinations of methodologies in sample collection, extraction, fractionation, and purification were also considered.

The mass spectrometer is a highly sensitive analyzer applicable for the detection of trace level analytes in a complex mixture.[14] Combining gas chromatography or liquid chromatography with MS offers the possibility of taking advantage of both chromatography as a highly resolving separation technique and MS as a powerful and sensitive detection and identification technique.[15] The combination gives a two-dimensional identification consisting of both a chromatogram retention time and a specific mass spectrum for each component in a mixture.[16] The SIM is one of the most versatile and commonly used spectrometric techniques. It allows the instrument to dwell for a greater proportion of the analysis time on those analytes of interest, resulting in an increase in sensitivity. LC coupling to MS, particulaly to a tandem mass spectrometer (LC–MS/MS), plays an important role in the development of new analytical strategies and was used to investigate and clarify trace levels of key components in asphalt fumes. The technique measures a transition from a metastable precursor ion to a product ion. The increased selectivity derives from the additional dimension of MS/MS. The purpose of using the collision-induced dissociation at varying collision energies in this experiment was to characterize fragmentation pathways leading to characteristic fragmentation patterns that can be used to clarify selected analytes of interest in a complex exposure mixture.

In addition, the analytical reliability of a method also paralleled the degree of isolation of the interesting analytes from a complex mixture. The purification processes were necessary to remove interfering compounds before final instrument identification and quantification. The extent of sample purification required was inversely related to the relative abundance of the analyte in the sample. In these simulated paving asphalt fumes, PAHs were contained at ultratrace levels. Solid-phase extraction was used as an important technique in sample preparation of this complex mixture. The major advantages included the following: (1) purification time was significantly reduced, and (2) the processes could be programmed automatically and precisely. Additionally, proper calibration was essential for quantitative analysis. Sixteen priority PAHs standards were used to develop a calibration curve. An internal standard offers the additional benefit of optimizing the accuracy of the assay. This was achieved by choosing a stable PAH isotope (perdeuterium anthracene) that had nearly the same chemical and physical characteristics as those of the analytes. In this way, the relative recovery of the internal standard could account for losses of the analytes during sample preparation and detection processes.

Characterization of Asphalt Fume Fractions. To characterize asphalt fume fractions and to determine the consistency of the asphalt aerosol within the generation system, asphalt fume samples were collected and analyzed at various points throughout the generation system including the asphalt oven, kettle, generator, and chamber (Figure 2a). Asphalt fume samples collected from the oven and kettle were monitored occasionally to check for any leaks in that part of the system. Routine analyses focused on samples collocated from the generator and chamber. The test asphalt was representative of the type used by the paving industry throughout the midwestern region of the United States. The asphalt was initially preheated in an oven, pumped to a large bitumen kettle, and then transferred it to the generator. The fume was then conducted from the generator to an exposure chamber through a heated transfer line. The efficiency of asphalt fume aerosol transfer to the exposure chamber was greatly improved

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by heating the transfer line between generator and chamber and designing the transfer line as short as possible. Two sampling trains were tested (Figure 2b) to compare sampling efficiency. The first train employed sampling through a 0.45-μm pore size TPFE filter, followed by a −190 °C cold trap of the volatile vapor fraction. Another sampling train consisted of a PTFE filter (0.45-μm pore size), an XAD-2 trap, and a charcoal trap in series at a flow rate of 1.0 L/min. The trapping efficiency of XAD-2 and charcoal traps proved to be as good as the cold trap. No significant difference was observed under the same experimental settings. The XAD-2 and charcoal traps were used throughout the experiments because XAD-2 and charcoal traps were easier to handle than the cold trap.

The sample treatment involved extraction, filtration, and preconcentration. Ultrasonic extraction was used to extract asphalt fume from collection media. It is a rapid, reproducible, and highly efficient technique for achieving solvent extraction. In operation, samples of either PTFE filter, XAD-2, or charcoal absorbents were transferred to separate PTFE tubes (30 mm). Dichloromethane/hexane (50% for each) was added into each tube with 5 mL per filter, per XAD-2 trap, or per charcoal trap. Ultrasonic extraction was performed for 1 h for filter samples and 1.5 h for XAD-2 and charcoal samples using a FS-220 ultrasonicator (320 W). After desorption of asphalt fume from the collection media, the extract solution was filtered into a test tube (16 mL) by a BenchMate II workstation solid-phase extractor, which was programmed to perform procedures automatically. Each of these fractions was carefully concentrated under a nitrogen stream. Samples were not evaporated to dryness to avoid loss of the very volatile components. Positive chemical ionization of GC/MS using methane produced a mass spectrum that was evaluated to characterize asphalt fume samples. The characteristic feature of using CI is that it is a soft ionization.

The analytical results were recorded as profiles of the total ion chromatogram (TIC) over specific ranges of mass-to-charge (m/z) ratios for each filter, XAD-2, and charcoal fraction. Total ion chromatograms acquired are displayed in Figure 3. The composition of asphalt fume can be grouped by considering the m/z values of major molecular ions. In Figure 3a, the TIC profiles present the asphalt fume composition of filter fractions for 3 consecutive days. Major components were observed over a range of molecular size of m/z 173−309 and eluted from the capillary column at retention times (RT) of 17−40 min. The plots of Figure 3b present the composition over a range of molecular size of m/z 121−197 at RT of 6−23 min for XAD-2 trap samples collected over 3 consecutive days. Figure 3c shows the composition of charcoal traps and the TIC profiles over a range of molecular size of m/z 57−141 at RT of 0.5−10 min for 3 consecutive days. Relative standard deviation for these trials ranged between 15.5 and 20.3%, which illustrated the reasonably good reliability and repeatability of the generation system. These results demonstrate, experimentally, that asphalt fume fractions can be characterized into three groups: (1) filter collecting a larger molecular size fraction; (2) XAD-2 trapping a medium molecular size fraction; and (3) charcoal trapping a small molecular fraction containing volatile vapor. These results also indicated that the generation system is capable of producing consistent test atmospheres of asphalt fume for inhalation toxicity studies under simulated road paving operation conditions.

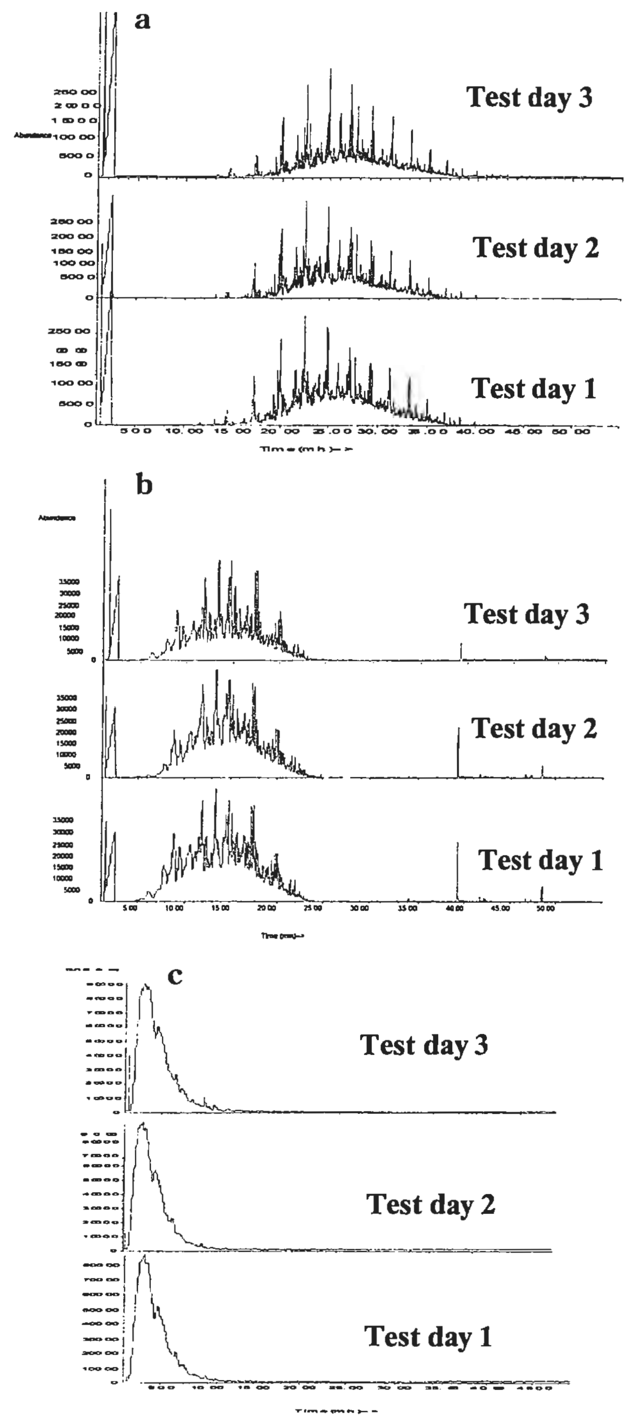

Figure 3. (a) Total ion chromatograms of asphalt fume filter fractions acquired by positive CI of GC/MS. (b) Total ion chromatograms of asphalt fume XAD-2 fractions acquired by positive CI of GC/MS. (c) Total ion chromatograms of asphalt fume charcoal fractions acquired by positive CI of GC/MS.

Quantification of Total Organic Matter of Asphalt Fumes. In the development of quantification procedures, the positive electron ionization mode of GC/MS was used. The sensitivity gained by positive EI allowed effective quantification of total organic matter in asphalt fumes. Quantitative applications of mass spectrometry were based on comparison between the ion current

Table 1. Determined Total Organic Matter (TOM) of Asphalt Fume Samples

asphalt exposure day[a]	asphalt fume collected by filter (mg/m^3)[b]	 trapped by XAD-2 (mg/m^3)	 trapped by charcoal (mg/m^3)	contents of TOM (mg/m^3)	RSD of TOM ($N = 5$)[c]
1	30.352	39.344	3.744	73.44	0.16
2	31.312	57.248	4.344	92.90	0.12
3	30.864	34.032	6.804	71.70	0.16
4	34.336	59.430	3.912	97.68	0.12
5	35.024	41.424	5.604	82.05	0.14

[a] Five typical exposure days, 3.5 h/day. [b] Contents of asphalt fumes calculated from calibration of a mixture of PAHs. [c] RSD, relative standard deviation from 5 days of collection.

obtained from the analytes and the ion current obtained from the standard. After performing maximum sensitive tuning, the estimated EI detection limit for typical priority PAH was 5.0 ng in full-scan mode. The GC/MS instrument was calibrated by a mixture of 16 priority standard PAHs and an internal standard of perdeuterium anthracene in the splitless mode. Total ion chromatograms were acquired using a temperature program from 50 to 310 °C by increasing temperature at a rate of 5 °C/min. A calibration curve was developed by multipoint measurements. The linear dynamic range exhibited by a mixture of PAHs was estimated over ~2 orders of magnitude (10–1000 ng). In this case, the coefficient of determination, r^2, was higher than 0.99 for the calibration curve.

To evaluate the recovery of the sample, stable isotope dilution with perdeuterited anthracene as an internal standard was used. The relative recovery of the internal standard can account for losses of the analytes during sample preparation and detection processes. This procedure offered an accurate calculation of relative total organic matter contained in asphalt fumes, allowed for the quantitation of the experiment, and improved the reliability of the analysis. In these experiments, perdeuterited anthracene was added to the sample at a final concentration of 100 ng/μL. The recovery of spiked PAHs on filter was 80%, and slightly lower recoveries were observed for XAD-2 and charcoal adsorbents. Quantitative results were based on calculations for a calibration curve of a mixture of 16 PAHs. The results of experimentally estimated total organic matter of asphalt fumes are displayed in Table 1 over 5 typical test days. The second column of Table 1 lists the organic matter of asphalt fumes collected on filters that range from 30.352 to 35.024 mg/m^3. The third column shows the organic matter of asphalt fumes trapped on XAD-2 tubes that range from 34.032 to 59.430 mg/m^3. The fourth column displays the organic matter of asphalt fumes trapped on charcoal tubes that range from 3.744 to 6.804 mg/m^3. The levels of estimated total organic matter of asphalt fumes are summarized in fifth column and range from 71.7 to 97.68 mg/m^3. The last column lists the relative standard deviation of five test days that range from 12 to 16%.

Identification and Quantification of Individual Priority PAHs. One of the objectives of this study was to identify and quantify individual priority PAHs in the laboratory-generated asphalt fume as potential source-specific indicators of exposure. Therefore, there is a need for a highly sensitive and selective chemical characterization technique that is capable of working with extremely small amounts of analytes containing in a complex mixture. Selected ion monitoring allows the mass spectrometer to detect specific compounds with high sensitivity. In SIM mode, only a few interesting masses are monitored with the attendant increase in sensitivity. This procedure was used and found to be suitable for this application.

Since the PAHs were contained at ultratrace levels, a reliable analytical procedure was used to isolate and purify specific priority PAHs from asphalt fume samples. A combination strategy of solvent extraction, solid-phase purification, and chromatographic separations was considered to achieve isolation of the PAHs of interest from asphalt fume samples. A BenchMate II workstation was employed to perform sequentially two purification stages. The use of SPE CN-U and SI provided the opportunity to remove polar components. After extraction of asphalt fume from the collection medium, the extract was transferred with a Pasteur pipet to a smooth-walled test tube (16 × 100 mm). This tube was loaded into the sample position of a BenchMate II workstation. The first purification stage was performed by a Bond Lut CN-U cartridge, which was conditioned with 3 mL each of methanol and hexane. A total of 3–6 mL of the sample was eluted through the cartridge and collected. An additional 1 mL of hexane was added to the cartridge, eluted, and combined with the same collection tube. The second purification stage was performed using a Bond Lut SI silica cartridge. The SPE performance for SI was the same as that for the CN-U cartridge. The extracts were filtered through a PTFE filter and preconcentrated by evaporating the solvent under a nitrogen stream using a TurboVap LV evaporator.

Selected ion monitoring of GC/MS was used to achieve maximal detection sensitivity after PAHs were isolated from the mixture. During the initial step of developing a quantitative procedure, standard quantities of the analyte were introduced into the mass spectrometer, and the instrument response was recorded. This process helped select a procedure that would give maximal sensitivity. It also permitted assessment of whether the analyte was susceptible to adsorptive losses at the sample-handling interface. In this case, the GC/MS was calibrated with individual priority PAHs separately and the internal standard of anthracene-d_{10} to provide a convenient and safe calibration. Three major ions listed in Table 2 were monitored for each specific PAH. Mass chromatograms were acquired using a temperature program from 50 to 310 °C by increasing temperature at a rate of 5 °C/min with a HP-5 MSD capillary column. After performing the maximum sensitive tuning, the detection limits for priority PAHs ranged between 0.03 and 0.18 ng/μL in the SIM mode (signal-to-noise ratio larger than 3:1). Three typical selected ion spectra of individual priority PAHs detected from asphalt fume filter samples are displayed in Figure 4. As illustrated, the ratios of three major ions from asphalt fume samples were observed to be similar to those from standard PAH solutions. It must be emphasized that this was one of the important criteria for identification. Further examination of the peak purity of the spectrum extracted by expected molecular ion mass indicated only a single component present, and it matched the library search results as illustrated in Figure 4. In addition, a peak in the characteristic ion current profiles at the expected retention time is good evidence for the

Table 2. Estimated Contents of Priority PAHs from Asphalt Fume Filter Samples

PAHs from asphalt fume samples	major ions to be monitored by GC/MS[a]	PAHs (ng/m^3)[b]	RSD ($N = 3$)[c]
naphthalene	128, 102, 64	1.15	0.26
acenaphthylene	152, 76, 63	6.93	0.34
2-bromonaphthalene	127, 101, 63	22.02	0.52
acenaphthene	154, 153, 76	18.65	0.53
fluorene	166, 139, 83	34.95	0.89
phenanthrene	178, 89, 76	57.53	0.78
anthracene	178, 89, 76	45.89	0.47
fluoranthene	202, 101, 88	20.48	0.54
pyrene	202, 101, 88	140.21	0.05
benzo[*a*]anthracene	228, 114, 101	53.49	0.16
chrysene	228, 114, 101	115.67	0.13
benzo[*b*]fluoranthene	252, 126, 113	22.04	0.08
benzo[*a*]pyrene	252, 126, 113	14.72	0.16
indeno[123-*cd*]pyrene	276, 138, 124	5.92	0.62
dibenzo[*a,h*]anthracene	278, 139, 124	12.25	0.28
benzo[*ghi*]perylene	276, 138, 124	22.76	0.30

[a] PAHs detected by SIM of GC/MS for three major ions of each component. [b] Contents of PAHs estimated by standard PAH calibrations with deuterated anthracene as an internal standard. [c] RSD, relative standard deviation of filter collections from 3 days, 3.5 h/day.

presence of the compound of interest. The retention times of monitored individual priority PAHs from asphalt fume samples were observed to be the same as those from the standard solutions under the same GC/MS instrumentation settings and conditions. Thus, it supports the conclusion that the trace levels of priority PAHs were contained in these simulated road paving asphalt fumes.

A quantitative relationship based on SIM calibration was established, and an internal standard multipoint calibration technique was used. The compounds were quantified using the ratio of the analyte and internal standard response. The linear dynamic range exhibited by a typical PAH was over a range greater than ~2 orders of magnitude (1.0−800 ng). In all cases, regression coefficients were between 0.98 and 0.99 for all the calibration curves. Quantification was calculated based on selected ion monitoring calibration curves. One standard PAH correspondingly calibrated one target analyte in the asphalt fume sample. Table 2 shows experimentally quantified priority PAH contents for 3 typical exposure test days with the selected ion monitoring technique. The second column of Table 2 lists the three major ions that were monitored for each PAH. The third column shows the calculated contents of 16 detected PAHs in filter fractions; the contents ranged between 1.15 and 140.21 ng/m^3. The fourth column shows the relative standard deviation of detection over a range of 16−89%. These results illustrate that a trace level of priority PAH can be detected in simulated road paving asphalt fumes. The method was highly sensitive and selective. These results also indicate that the contents of individual priority PAHs in asphalt fumes vary greatly at trace levels for samples collected from day to day. Asphalt fume may contain non-PAH hydrocarbons in great excess.

Clarification of Key Components by Microflow LC-Q-TOF MS. Although the selected ion monitoring GC/MS method described above has been used to assess the individual priority PAHs in asphalt fume samples, an advance technique of microflow LC coupled to Q-TOF MS was used to further investigate and clarify trace levels of key components in asphalt fumes. This system offers some advantages over GC/MS in certain mixture analysis applications. The technique of MS/MS measures a metastable transition or reaction from a metastable precursor ion to a product ion. Monitoring a metastable decomposition reaction provides a means by which to increase the selectivity of an assay for a given analyte. The increased selectivity derives from the additional dimension of MS/MS. The operational features in this system consist of a standard Z-spray source fitted with a corona discharge pin and a heated nebulizer probe. An APCI source was employed for this analysis, because electrospray exhibits inefficient ionization for this application. The mobile phase from the LC column entered the probe where it was pneumatically converted into an aerosol and was rapidly heated and converted to a vapor/gas at the probe tip. The Q-TOF mass spectrometer was operated in the positive APCI mode. The analyte molecules, introduced into the mobile phase, reacted with the reagent ions at atmospheric pressure and produced single-charged protonated molecules for a broad range. Source temperature was set at 120 °C, and the APCI probe temperature was maintained at 400 °C. The scan rate was set at 1.0 scan/s. The APCI drying gas was nitrogen set at 400 L/h. Many instrument parameters, besides probe temperature, source temperature, and cone voltage, influence the tuning maximal sensitivity and resolution. They were all optimized and held constant during each analysis.

Following the optimization procedure, a mobile phase of 50% dichloromethane and 50% methanol was used to elute the asphalt fume sample from a LC column (Nucleosil C_{18} PAH column, 5 μm, 1000-μm i.d.) at microflow rate of 60 μL/min. An aliquot of sample was injected onto the analytical column using a LC packing Famas autoinjector. The mass spectrometer was adjusted to a resolution of ~10 000. The estimated detection limit of microflow LC−Q-TOF MS for PAH at microflow rate of 60 μL/min with a mobile phase of 50% dichloromethane and 50% methanol was less than 0.5 pmol (signal-to-noise ratio larger than 10:1). The operation of the TOF mass spectrometer was ideally suited for selected ion monitoring. In principle, the competing ionization mechanisms in positive APCI are proton transfer to form $[M + H]^+$ and charge transfer to form $M^{+\cdot}$ species.[17] Using above-described experimental conditions, the greatest ion intensities were observed through proton transfer in these studies. All operational parameters were optimized and held constant throughout the experiment except the collision energy, which was varied for each specific run. In operation of the Q-TOF MS for MS/MS, a precursor ion spectrum was produced by scanning the first quadrupole, and a product ion spectrum was produced by holding the first quadrupole at constant m/z while scanning the TOF MS. An autoswitch from MS to MS/MS mode was used in this experiment.

The precursor ion m/z 253 for detection of benzo[*a*]pyrene with a single charge state was selected. A MS/MS fragmentation was automatically turned on when the selected precursor ion intensity threshold (counts/s) was larger than 10, and then MS/MS was automatically turned off when the intensity fell below a threshold of 10. Variation of collision-induced dissociation energies offered great advantages in the identification of selected PAHs.

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(17) French, J. B.; Davidson, W. R.; Reid, N. M.; Buckley, J. A. In *Tandem Mass Spectrometry*; McLafferty, F. W., Ed.; Wiley-Interscience: New York, 1993; pp 353−370.

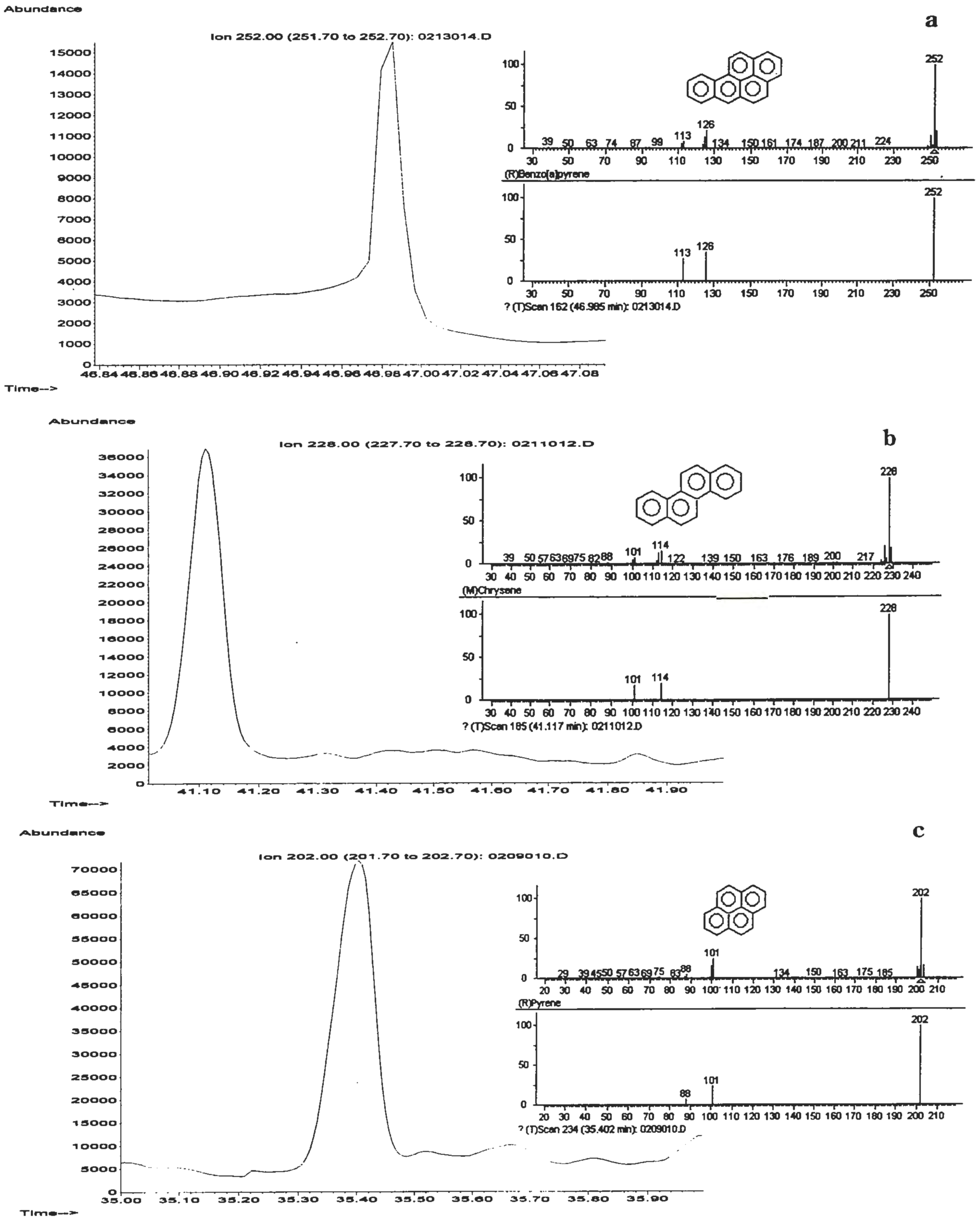

Figure 4. (a) Benzo[*a*]pyrene detected from an asphalt fume filter fraction by selected ion monitoring GC/MS. (b) Chrysene detected from an asphalt fume filter fraction by selected ion monitoring GC/MS. (c) Pyrene detected from an asphalt fume filter fraction by selected ion monitoring GC/MS.

During collision-induced dissociation, the ion under investigation was chosen as the precursor ion in the first MS of the quandrupole and directed into a collision cell, where it collided with a collision gas to acquire internal energy, which lead to its decomposition into product ions and detection by the second MS. The detection of Bap from asphalt fume samples was performed by optimizing

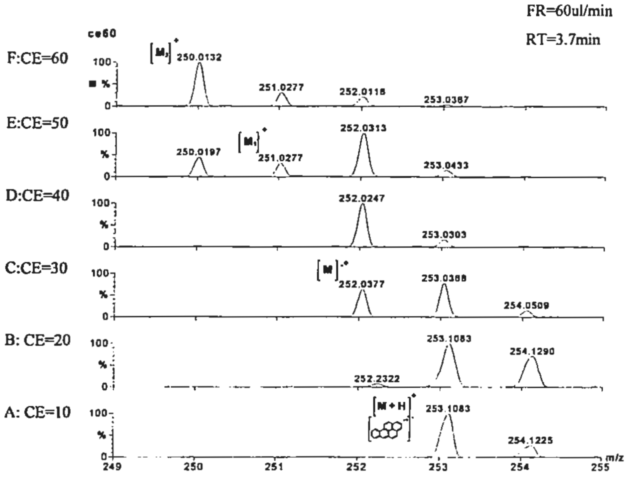

Figure 5. MS/MS spectra of a benzo[*a*]pyrene characteristic fragmentation pattern acquired from an asphalt fume filter fraction. The spectra obtained with variation of collision-induced dissociation energy from 10 to 60 by a microflow LC/Q-TOF MS. Source temperature, 120 °C; APCI probe temperature, 400 °C; scan rate, 1.0/s; APCI drying gas, nitrogen at 400 L/h.

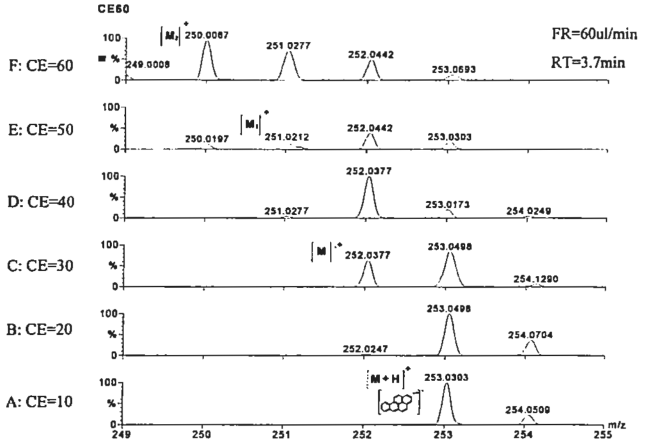

Figure 6. MS/MS spectra of a benzo[*a*]pyrene characteristic fragmentation pattern acquired from a standard benzo[*a*]pyrene solution. The spectra obtained with variation of collision-induced dissociation energy from 10 to 60 by a microflow LC/Q-TOF MS. Source temperature, 120 °C; APCI probe temperature, 400 °C; scan rate, 1.0/s; APCI drying gas, nitrogen at 400 L/h.

instrument settings with variation of collision energy from 10 to 60. Great selectivity was gained using high-resolution TOF at a MS/MS mode to obtain characteristic fragmentation patterns for selected target analytes as shown in Figure 5. Figure 6 displays the fragmentation pathway of standard Bap. In comparison to these spectra, it was observed that the greatest ion intensity products of Bap through proton-transfer ions $[M + H]^+$ presented in both Figure 5a (*m/z* 253.0303) and Figure 6a (*m/z* 253.1083) were at a collision energy of 10. At this stage, an important feature of these two plots was that no charge-transfer product ions were observed. However, when the collision energy was increased to 30, a significant charge-transfer product ion $[M]^{\cdot +}$ (*m/z* 252.0377) was observed from both pathways. One pathway was obtained from asphalt fume filter fractions in Figure 5c and another one was acquired from standard Bap solutions in Figure 6c. They were observed at exactly the same *m/z* values. Specifically, the intensity ratios of charge-transfer product ions $[M]^{\cdot +}$ to the proton-transfer product ions $[M]^{+\cdot}/[M + H]^+$ detected from the asphalt sample in Figure 5c–f were approximately equal-to-those ratios from standard Bap solution in Figure 6c–f. At the high collision-induced dissociation energy of 60, a proton-transfer ion was greatly decomposed, and the predominant products ion were fragment ions as observed in both Figure 5f ($[M_2]^+$ *m/z* 250.0132) from asphalt fume sample and Figure 6f ($[M_2]^+$ *m/z* 250.0067) from the standard Bap solution. All of these spectra were acquired by varying collision-induced dissociation energies from 10 to 60. By interpretation of the spectra in both Figure 5 and Figure 6, the presence of Bap in this simulated asphalt fume filter fraction could be clearly verified. The results demonstrate that the collision-induced dissociation process used to characterize fragmentation pathways and to obtain characteristic fragmentation patterns can be used to identify selected key analytes in complex mixed exposure samples.

High-resolution Q-TOF MS was also tested in an attempt to observe the complex nature of asphalt fumes. Q-TOF mass spectrometer instrument parameters including probe temperature, source temperature, TOF voltage, and cone voltage were optimized and held constant during sample analysis. Figure 7 displays a typical mass spectrum acquired by a constant infusion (5 μL/min) of an asphalt fume filter sample into the APCI source. The mass spectrum is given in Figure 7. Hundreds of molecular ion peaks were present, indicating that hundreds of components were observed in asphalt fumes. Thus, asphalt fume exposure is a complex mixed exposure.

SUMMARY

A highly sensitive, selective, and reliable analytical method has been developed and validated for the characterization of asphalt fume composition and determination of the consistency of the aerosol produced by a fume generator. Using positive chemical ionization, the composition of asphalt fumes could be characterized into three fractions: (1) filter collection of a larger molecular size fraction over a range of *m/z* 179–309; (2) XAD-2 trapping of a medium molecular size fraction over a range of *m/z* 121–197; and (3) charcoal trapping of a small molecular size vapor fraction over a range of *m/z* 57–141. Sixteen individual priority PAHs, which included naphthalene, acenaphthylene, 2-bromonaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, indeno[123-*cd*]pyrene, dibenz[*a,h*]-anthracene, and benzo[*ghi*]perylene, were monitored and identified at trace levels in the simulated asphalt fume samples. These results may contribute to an improved understanding of the biological effects of asphalt fume exposure.

Analytical methodologies described in this study covered several specific advanced mass spectrometric techniques. Programmable SIM offered an optimal compromise between operating the GC/MS and microflow LC/Q-TOF MS for maximum sensitivity and obtaining high reliability of analytical results. The reported approach of using a collision-induced dissociation process to characterize fragmentation pathways leading to obtain characteristic fragmentation patterns represents a new approach to identify potentially hazardous components in complex mixtures. The advancing technology of hybrid Q-TOF is just beginning to add much needed analytical power to our arsenal for characterization. These developments present an important methodology,

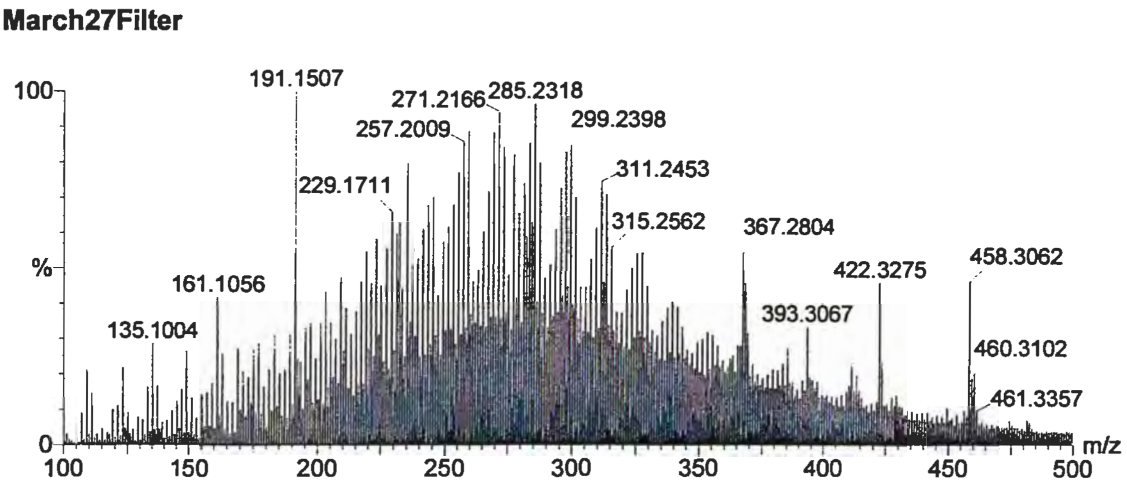

Figure 7. Typical high-resolution mass spectrum of an asphalt fume filter fraction characterized by APCI of Q-TOF MS.

which accelerates the integration of mass spectrometry into work-related research strategies.

These results indicate that the asphalt fume generation system was capable of producing a consistent test atmosphere of asphalt fume under simulated road paving conditions for inhalation toxicity studies. In comparison to many traditional detection techniques, the methods developed in this study were highly sensitive, selective, and reliable. They are adaptable for occupational and environmental applications. Future studies will focus on method development for assessment of initial biomarkers to predict exposure dose and to develop a link between exposure to key component/chemical classes and adverse biological effects in occupational mixed exposure studies.

ACKNOWLEDGMENT

This research work was funded, in part, by an interagency agreement with NIEHS/NTP. Thanks are also extended to Al Munson, Douglas Sharpnack, Nancy Bollinger, Paul H. Nicolaysen, Jean Meade, Judy Mull and Beverly Carter for their project support and coordination. Jane Ma and Chris Coffey provided critical review of the manuscript.

Received for review March 20, 2001. Accepted May 24, 2001.

AC010334M

Characterization of Asphalt Fume Composition under Simulated Road Paving Conditions by GC/MS and Microflow LC/Quadrupole Time-of-Flight MS

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ANALYTICAL® CHEMISTRY

Reprinted from
Volume 73, Number 15, Pages 3691–3700