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Trueness, Precision, and Detectability for Sampling and Analysis of Organic Species in Airborne Particulate Matter

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Abstract

Recovery, precision, limits of detection and quantitation, blank levels, calibration linearity, and agreement with certified reference materials were determined for two classes of organic components of airborne particulate matter, polycyclic aromatic hydrocarbons and hopanes using typical sampling and gas chromatography/mass spectrometry analysis methods. These determinations were based on initial method proficiency tests and on-going internal quality control procedures. Recoveries generally ranged from 75% to 85% for all target analytes and collocated sample precision estimates were generally better than 20% for polycyclic aromatic hydrocarbons and better than 25% for hopanes. Results indicated substantial differences in data quality between the polycyclic aromatic hydrocarbons and hopanes. Polycyclic aromatic hydrocarbons demonstrated better collocated precision, lower method detection limits, lower blank levels, and better agreement with certified reference materials than the hopanes. The most serious area of concern was the disagreement between measured and expected values in the standard reference material for hopanes. With this exception, good data quality was demonstrated for all target analytes on all other data quality indicators.

Introduction

A better understanding of measurement uncertainties for organic species in airborne particulate matter (PM) is a critical research need. Airborne PM remains a serious public health issue [1] and PM organic components play an important role in PM health effects [2], source apportionment [3,4], and air quality modeling [5]. Despite widespread use of solvent extraction and gas chromatography/mass spectrometry (GC/MS) methods for analysis of PM organic species, a comprehensive assessment of data quality following International Union of Pure and Applied Chemists (IUPAC) and EPA recommended method validation guidelines [6,7] under instrument operating conditions during analysis of field samples has not been previously reported.

This paper describes trueness, precision, limits of detection and quantitation, blank levels, calibration linearity, and analysis of certified reference materials or other control samples as recommended by IUPAC and EPA for a combination of typical sampling and analysis methods for PM organic speciation analysis. Results will be of interest to environmental, exposure and health scientists with need of more realistic estimates of precision and bias for airborne PM organic species. The results will also be useful for research planning that requires information on expected uncertainties and detectability. In addition, the paper can be viewed as a model for applying IUPAC and EPA method validation recommendations to PM organic speciation methods and for developing realistic data quality objectives for analysis of PM organic species.

Historically, characterization of method performance and overall uncertainties associated with organic speciation measurements has proved difficult, in part because of the lack of available standards or certified reference materials. As a result, overall measurement uncertainty estimates are often not available. For example, in early PM source apportionment modeling

efforts with organic species, overall uncertainty estimates were based on a single method component (GC/MS analysis only) precision [8-10]. Method recovery estimates in these studies have typically been based on representative model compounds [11], rather than actual target analytes. More recently, collocated sampling precision [12], method detection limits [13], blank levels [14], and method intercomparisons [15] have been described, but GC/MS analysis of PM organic species is still largely viewed as a research method, with accordingly limited requirements for quality assurance procedures or method performance testing. The extensive database of quality control sample results from a recent major human exposure field study, the Detroit Exposure and Aerosol Research Study [16], along with newly available certified reference materials relevant for PM organic analysis, including hopanes and steranes (SRM 2266), provided a unique opportunity to evaluate key data quality indicators for PM organic speciation sampling and analysis.

Method

Figure 1 describes the combined sampling and analytical method. A total of 40 samples (58 including analytical duplicates and 88 including quality control samples) were collected on quartz fiber filters (previously baked for 4 hours at 550 °C) using a Tisch TE-1202 sampler operating at a nominal flow rate of 113 liters per minute [14]. Target analytes were solvent extracted, concentrated under purified nitrogen, prepared for analysis by solid phase extraction (SPE), and analyzed by GC/MS with selected ion monitoring (SIM).

Table 1 shows the target compounds, deuterium-labeled surrogates, internal standards, and their quantitation ions used for determining concentrations and recoveries. Target compounds included PAHs and hopanes. PAHs are among the most frequently analyzed species

in airborne PM because of their potential carcinogenic and inflammatory properties. Hopanes are among the most useful diagnostic tracers for automobile exhaust PM, which is often a major local source of urban PM mass. Individual PAH and hopane species were selected as target compounds based on their abundance and detectability in typical urban PM samples. Other compounds are known to coelute with three of the target compounds on the DB-5 column. Triphenylene coelutes with chrysene, benzo[j]fluoranthene coelutes with benzo[b]fluoranthene, and dibenz[a,c]anthracene coelutes with dibenz[a,h]anthracene. In these cases concentrations are quantified using the calibration curve for the specified target compound, but reported as the sum of both coeluting compounds. Chemical materials used in target, surrogate, and internal standard solutions preparation were obtained from Absolute Standards, AccuStandard, Chiron, Cambridge Laboratories, and the National Institute of Standards and Technology (NIST). Organic solvents were purchased from Burdick & Jackson and were of either GC^2 or High Purity grade quality.

Extraction

Prior to extraction, filter samples were spiked with the deuterium-labeled surrogate analytes listed in Table 1 to monitor recovery. Surrogate analytes were spiked to produce a final concentration of 500 pg/μL at 100% recovery. Due to the unavailability of deuterium-labeled hopane materials, n-triacontane-d62 functioned as the surrogate for the hopane species.

Samples were extracted with equal parts, by volume, hexane, methanol, and dichloromethane using a Dionex ASE 200 Accelerated Solvent Extractor at 100 °C in 11 mL extraction cells pressurized to 100 atm. This solvent mixture was selected based on its previous performance in extracting compounds over a wide polarity range from airborne PM [17], which is critical to our long-term research program to apply organic markers in PM source

apportionment. Each sample extraction cell was extracted for two five minute static cycles using a 100% flush volume and 60 second high purity nitrogen purge. Following the extraction, the sample extracts were reduced to approximately 3 mL using a Zymark TurboVap LV Evaporator with high purity nitrogen purge. The samples were then quantitatively transferred to 5 mL concentrator tubes and further reduced to 100 µL under a stream of high purity nitrogen.

Solid Phase Extraction Cleanup (SPE)

Cleanup by solid phase extraction (SPE) was found to be necessary since severe column deterioration developed following the analysis of only a few samples, resulting in poor peak shape and calibration verification failure [18]. SPE cleanup was carried out using Supelco custom 3-mL glass Supelclean LC-Si SPE cartridges equipped with Teflon frits and loaded with 500 mg of silica (45 µm particle size, 0.8 cm^3/g pore volume, 60 Å pore size, 475 m^2/g surface area). Concentrated sample extracts were quantitatively transferred to the SPE cartridges and target analytes eluted in a mixture of 1% dichloromethane and 1% acetone in hexane using a Supelco Visiprep DL SPE Vacuum Manifold system [18]. Five 1-mL fractions were collected in a 5 mL concentrator tube. The collected fractions were then reduced to a 100 µL volume under a stream of high purity nitrogen using the circulating water bath. Each SPE processed sample was spiked with a mixture of the deuterium-labeled internal standards listed in Table 1 and diluted to a final volume of 300 µL with dichloromethane to produce a final internal standard concentration of 1000 pg/µL in a solution comprised of approximately 60% dichloromethane and 40% hexane. All samples were stored at -80°C prior to analysis in vials capped with double-faced Teflon silicone septa. These caps were replaced with pure Teflon septa for injection and immediately replaced with the original septum following injection and returned to storage. Precision results

from analytical duplicates indicate no changes in concentration due to vial opening or waiting on the auto-injector between removal from storage and injection.

Identification and Quantification

Qualitative identification was based on the retention time of the authentic target analyte standard's quantitation ion and on the relative abundance of the quantitation and qualifier ions listed in Table 1. GC/MS analysis was carried out with an Agilent 6890 GC with auto-injector using splitless injection and an Agilent 5973 Mass Selective Detector (MSD) with high temperature inert ion source upgrade operated in selected ion monitoring (SIM) mode. A splitless injection with pressure pulse of 30 psi and pulse hold time of 0.70 minutes was used to minimize flashback and molecular weight discrimination. Auto-injections of 1.0 µL were conducted at an inlet temperature of 320°C and purge time of 0.50 minutes using an Agilent single-taper, deactivated inlet liner without glass wool. The MS transfer line temperature was maintained at 320°C. The MSD was optimized for maximum mass ion signal response and operated at source and quadrupole temperatures of 300°C and 200°C, respectively. Chromatographic separation was accomplished with a J&W DB-5MS, 30m, 0.25mm id, 0.25µm film capillary column with He flow at 1 mL/minute. GC separation conditions are listed as follows: 50°C for 1.25 min., to 160°C at 15°C/min. hold 2 min., to 220°C at 3.5°C/min. hold 2 min., to 265 °C at 2°C/min. hold 2 min., to 300°C at 1.5°C/min., to 315°C at 20°C/min. hold 5 min.

Atmospheric concentrations were determined from calibration curves established for each target compound with a minimum of four concentration levels. An average relative response

factor using all calibration solutions was determined for all target and surrogate analytes using Equation 1:

$$RRF = \frac{A_x C_{is}}{A_{is} C_x} \qquad (1)$$

where RRF is the relative response factor, X is the measured analyte, IS is the internal standard, A is the integrated area of the chromatographic peak, and C is the concentration injected. Internal standards were added in known amounts to the final extract immediately following all sample preparation steps, an approach that is widely used in environmental analysis [19]. Using this approach, a consistent internal standard concentration was maintained between samples, with inefficiencies and losses occurring during extraction and sample processing accounted for using deuterium-labeled surrogate analytes added prior to extraction.

Concentrations were determined with Equation 2:

$$\frac{A_x}{A_{is}} = (\text{slope})\frac{C_x}{C_{is}} + (\text{intercept}) \qquad (2)$$

where the slope and intercept are determined from the linear regression of A_x/A_{is} against C_x/C_{is} for all concentration levels used to establish the calibration curve. Atmospheric concentrations in picograms per cubic meter were determined by multiplying C_x in picograms per microliter by the total volume of the extract in microliters and dividing by the total amount of air sampled in cubic meters.

Initial Method Proficiency Test

Recoveries of all target analytes were determined in triplicate at three concentration levels to provide an initial indication of recovery and precision. Clean filter blanks were also

evaluated with no departure from baseline observed for any of the target analytes. In addition, Method Detection Limits (MDL's) and Limits of Quantitation (LOQ's) were also determined for all target analytes.

Quality Control

A method blank, a matrix spike, and duplicate sample were analyzed for each of 10 batches consisting of approximately eight samples. A method blank was a previously baked quartz fiber filter used for monitoring background contamination of target analytes and measuring percent recovery of the deuterium-labeled surrogate analytes listed in Table 1. The matrix spike was a previously baked quartz fiber filter used to measure percent recovery of both the deuterium-labeled surrogate and target analytes. Field blanks were prepared by installing a blank filter holder inside a static air sampler for the designated sampling period. Trip blanks were filter holders containing blank filters that traveled to and from the sampling destination, without installation in the sampler [14]. Collocated samples were collected from two samplers located at the same sampling site. Prior to extraction, each method blank, matrix spike sample, and filter sample (including field blanks and trip blanks) was spiked with the deuterium-labeled surrogate analytes shown in Table 1 at a concentration of approximately 500 pg/μL assuming 100% recovery. The matrix spike sample was spiked with the PAH and hopane analytes at concentrations of approximately 70 pg/μL and 100 pg/μL, respectively, assuming 100% recovery.

Selected calibration verification solutions, constituent solutions of the calibration curve, were analyzed following the injection of a maximum of three samples to verify calibration curve stability and accuracy during the analysis. Quality Control (QC) solutions, prepared

independently of the calibration curve solutions, were also analyzed and compared to their prepared values. When possible, standard reference materials (SRMs) acquired from the National Institute of Standards and Technology (NIST) were prepared as QC solutions for analysis. When a NIST source was not available, reference standards alternative to those used for the calibration solutions were selected from other vendors including Absolute Standards, AccuStandard, and Chiron.

Results

Concentrations and Ranges

Table 2 summarizes observed concentrations and ranges for ambient samples. For both the hopanes and most abundant PAHs the average concentrations ranged from approximately 100 pg/m^3 to 200 pg/m^3. Individual sample concentrations ranged from less than 100 pg/m^3 to several hundred pg/m^3. The highest average PAH concentrations were observed for benzo[g,h,i]perylene (202 pg/m^3) and benzo[b+j]fluoranthene (190 pg/m^3). The highest average hopane concentrations were observed for both 17α(H),21β(H)-30-norhopane and 17α(H),21β(H)-22S-homohopane, each at 150 pg/m^3.

Linearity

Calibration consisted of two components: 1) an initial calibration curve established using solutions containing known concentrations of internal standards, surrogates, and target analytes and 2) calibration verifications performed following the injection of three samples using selected solutions from the initial calibration curve. Linear calibration curves were established for each target analyte with a minimum of four concentration levels prior to the analysis of samples. R^2

values of ≥0.996 were achieved for all PAHs and ≥0.995 for all hopane species. Due to unexpected instrument maintenance the GC/MS was recalibrated following the analysis of the first sample batch. The percent relative standard deviation (RSD) of the average response ratio for the first batch ranged from 1.5% to 15% for all PAHs except benzo[a]pyrene (< 19%) and from 6% to 19% for all hopanes. For the remaining nine sample batches, the average response ratio RSD for all individual target compounds ranged from 1.5% to 14%.

In spite of these high correlation coefficients, considerable deviation between predicted and observed values was observed at the low end of the calibration curve if a linear calibration was applied across the entire range of concentrations typically measured for most compounds. This pattern, resulting from heteroscedasticity of calibration data, is common enough in analytical calibrations that the use of the correlation coefficient as an indication of quality of fit has been described as misleading and inappropriate as a test for calibration linearity, despite its widespread use [6]. This was a particular concern for these samples because a substantial number of target analyte concentrations were at the low end of the calibration curve. For this reason multiple linear calibration curves were established for different sample concentration ranges.

It was necessary to use three different curve ranges as shown in Table 3 to avoid the non-linearity effects caused by upper end bias of the curve. For example, for low concentration samples, a calibration curve over a small range of low concentrations was generated and applied, while for higher concentration samples, a curve range spanning higher concentrations was used. To ensure calibration stability, the next to lowest and next to highest calibration standards were analyzed after every three samples for each of the calibration curves used to quantify sample concentrations in those samples, and to verify that all concentrations were consistent with

prepared values. Table 3 shows the two calibration solution verification check results for three different calibration curves. For n number of replicates at each verification level, the average target concentration, percent relative standard deviation (%RSD), and percent difference between the average and prepared calibration verification solution is reported. A RSD of less than 10% was observed for each average target concentration level for all calibration verification replicates. Also, the average target concentration, for all calibration verification levels for all three curves, exhibits less than 10% difference from the prepared concentration for all but two targets and these two were within 15%. Two other calibration curves, spanning higher concentration ranges, were also used for the quantification of a single sample (one calibration verification check per curve). For both of these curves, the percent difference between the calibration verification check and the prepared value was less than or equal to 10% for all targets.

Recovery

Recovery, defined as 1-bias [20] is used as an alternative expression for bias [6] as a quantitative measure of trueness. Table 4 summarizes three separate estimates of recovery determined. First, recovery data measured in triplicate for three concentration levels in the initial method proficiency demonstration indicated sufficient quality for sample analysis to proceed. Second, matrix spike quality control samples provided an on-going assessment of target analyte recoveries under instrument and column conditions present during sample analysis and precision data between spiked batches. Third, surrogate analytes were used to assess the effect of the collected PM matrix on recovery, although this should be considered an upper limit because potentially, deuterium-labeled spiked materials can be more easily extracted than species

native to the PM [21]. Altogether the results provide an indication of recovery consistency that takes into account differences in instrument conditions and PM matrix effects.

Recoveries from the method proficiency evaluation were close to 80% with RSDs generally less than 10%. Matrix spike recoveries for all targets except pyrene ranged from approximately 75% to 85% with less than 20% RSDs for all PAHs and approximately less than or equal to 25% RSDs for the hopane species. The lower recovery for pyrene most likely reflects the volatility of a lower molecular weight four-ring PAH. Surrogate analyte recoveries ranged from approximately 75% to 85% with a variability of less than 15%. No statistically significant differences in recoveries were observed between initial method proficiency tests and matrix spikes, between target compounds in the matrix spikes and their corresponding deuterium-labeled surrogate sample spikes, or between recoveries at the highest and lowest concentrations for any compound (two tailed t-test, $\alpha = 0.05$). Method blank and matrix spike surrogate recoveries were also comparable to sample surrogate recoveries..

Precision

Analytical precision applies to GC/MS analysis only, and does not reflect sample collection, sample extraction, and sample processing conducted before GC/MS injection. To monitor analytical precision, duplicate sample injections were made from each batch of samples. For each sample pair, analytical variability was determined using Equation 3, where $C_{i,ha}$ and $C_{i,la}$, are the highest and lowest of the two sample analyses of concentration measurements for each sample i, respectively, $C_{i,avg}$, the average of the two sample measurements of samples i, and n, the total number of duplicate measurements taken.

$$p = \frac{\sum_i (C_{i,ha} - C_{i,la}) / C_{i,avg}}{n} \quad (3)$$

To estimate sampling and method precision, five collocated sample pairs were analyzed and evaluated using Equation 3. Collocated samples were collected from two samplers located at the same sampling site. Uncertainties associated with sample processing and GC/MS analysis are probably the greatest contributors to collocated sampling precision, rather than differences in the sampling process, and collocated precision should be considered an overall uncertainty estimate for the entire sampling and analysis process, including sample collection, sample processing (extraction, cleanup, etc.), and GC/MS analysis.

Table 5 summarizes the precision results. Analytical precision was generally within 5% for most PAHs and within 10% for all hopanes, demonstrating good agreement between duplicate injections. Sampling and method precision among the five collocated samples was approximately 20% or less for most PAHs and approximately 25% or less for all hopane species. These results are similar to those reported by Manchester-Neesvig et al. [12].

An independent propagated error evaluation was also conducted to assess the total process error associated with the season 2 sample analysis and was compared to the measurement based collocated precision estimate in Table 5. The total process was considered to consist of three unique categories: 1) sample collection, 2) sample extraction and processing, and 3) GC/MS analysis. The propagated error for each process category was determined and combined to produce a total error using acceptable statistical approaches for the propagation of error. Results show good agreement between collocated sampling precision that reflects uncertainty associated with the entire process, and the calculated propagated errors derived from

uncertainties associated with the individual sample collection, sample extraction and processing, and GC/MS analysis components.

Method Detection Limits

Table 6 shows Method Detection Limits (MDL) and Limits of Quantitation (LOQ) for each target analyte expressed as both pg/μL injected and pg/m^3 sampled as well as the percentage of samples with concentrations greater than LOQ. MDLs were determined for the entire analytical method, including extraction, SPE cleanup step, and GC/MS analysis following the "Definition and Procedure for the Determination of the Method Detection Limit", described in the Electronic Code of Federal Regulations, Title 40CFR136 Appendix B, Revision 1.11 [22], recommended for method validation. Following these recommendations, nine replicate 102 mm quartz fiber filters, prebaked at 550°C to minimize background contributions, were spiked with a target compound spiking solution to produce a post-method concentration between one and five times the estimated detection limit, processed through all procedural steps and analyzed by GC/MS.

MDLs were determined by multiplying the standard deviation of nine replicate samples by the corresponding 99% t value, t=2.896. If post method concentration results were outside the one to five times estimated detection limit range, spiking volumes were readjusted, and MDL determinations repeated for those compounds. If concentration results were outside the range for one or more individual samples (for benzo[a]pyrene, perylene, and indeno[1,2,3-cd]pyrene) MDL values were determined using the appropriate student t value consistent with the acceptable number of replicates determined for the affected target analyte. The LOQ is mathematically

defined as equal to 10 times the standard deviation for a series of replicates used to determine a justifiable limit of detection, the MDL (ACS 1980).

As seen from Table 6, MDLs ranged from approximately 0.3 pg/μL to 1.2 pg/μL for PAHs and from approximately 1.5 pg/μL to 3.0 pg/μL for hopanes. For 24 hour samples collected at 113 liters per minute, this corresponds to approximately 0.4 pg/m^3 to 2.9 pg/m^3 for the PAHs and from approximately 2.5 to 11 pg/m^3 for the hopanes. In part because selective ion monitoring was used, MDLs are considerably lower than those reported by Sheesley et al. [13]. In addition, Table 6 shows that the percent of samples greater than the limit of quantitation for most target analytes was 100%, with the exception of 17α(H),21β(H)-22S-homohopane, reporting 98%.

The original MDL determinations were carried out prior to the season 2 sample analysis. MDLs were also measured after the analysis of all 88 samples following the same original MDL procedure. Substantial increases were observed for the hopanes, with MDL's increasing by a factor of 2 for 17α(H),21β(H)-30-norhopane, by a factor of 3 for 17α(H),21β(H)-hopane, and by a factor of 4 for 17α(H),21β(H)-22S-homohopane. Such departures from original MDL determinations were not observed for the PAHs. MDL's increased by 40% to 60% for chrysene, picene, and benzo[k]fluoranthene, but by less than 30% for all other PAHs. To evaluate the statistical significance between the initial and after sample MDL determinations, an F-Test Variance Ratio for each target analyte was performed (two-tailed, $\alpha = 0.5$). Results confirmed no difference between the initial and after sample determinations for all target analytes excluding 17α(H),21β(H)-hopane. F-ratios for 17α(H),21β(H)-30-norhopane and 17α(H),21β(H)-22S-homohopane were higher as well, though not significant at 95% confidence. Poorer sensitivity is expected as the ion source becomes dirtier, the filament gets worn, or the electron multiplier ages

during analysis of a large number of samples. As a result, the hopanes appear to show slightly greater vulnerability to sensitivity loss over the course of the sample analysis process, resulting in higher after sample MDL results.

Blanks

Table 7 describes average concentration results for ten method blanks, five field blanks, and five trip blanks. Concentration averages are reported for only those filters showing measurable levels of target analyte. All method blank target levels were below MDL, an indication that the prebaking of blank filters is effective in removing residual organic contaminants. On average, all compounds for the field and trip blanks excluding pyrene, chrysene + triphenylene (field blank only), 17α(H),21β(H)-30-norhopane, and 17α(H),21β(H)-hopane were less than the MDL with most target components exhibiting no departure from baseline. Average field and trip blank levels for pyrene, chrysene + triphenylene (field blank only), 17α(H),21β(H)-30-norhopane, and 17α(H),21β(H)-hopane were greater than MDL but less than LOQ. The trip and field blank levels for pyrene, both at 0.7 pg/m^3 and the field blank level for chrysene + triphenylene at 0.63 pg/m^3, are only slightly above the MDL and most likely due to low level absorption realized during field and trip blank activities. This is consistent with measurable levels of pyrene observed in the method blanks at an average concentration of 0.1 pg/m^3.

The ubiquitous and variable nature of hopane species in lubricating fluids and vehicle emissions are likely sources contributing to the field and trip blank levels with the hopanes appearing to be more susceptible to contamination than the PAHs. However, the field and trip blank levels for the hopanes were still much lower than sample concentration levels, and hopanes

were not detected in all field and trip blanks. Because all sample hopane component concentrations were well above MDLs, the corresponding hopane component blank levels were no greater than 10% of the average sample concentrations as shown in Table 7. As a result, blank levels did not substantially compromise the quality of the data reporting for these components.

Traceability and Control

Quality Control (QC) solutions, prepared independently of the calibration curve solutions, were analyzed and compared to their predicted values. Table 8 presents the results for this analysis and shows the related accuracy and agreement between the QC and calibration solutions. When possible, standard reference materials (SRMs) acquired from the National Institute of Standards and Technology (NIST) were analyzed. When a NIST source was not available, reference standards alternative to those used for the calibration solutions were selected from other vendors including Absolute Standards, AccuStandard, and Chiron. Four QC solutions were prepared to evaluate performance over the entire calibration range. Results for one of the target analyte QC solutions prepared at approximately 100 pg/μL, are presented in Table 8 and are typical of all QC solution results.

Most PAHs are within 20% of prepared value except for benzo[k]fluoranthene, benzo[a]pyrene, and picene which differ by 24%, 23%, and 25%, respectively. Analysis of the remaining PAH QC solutions, ranging nominally in concentration from approximately 5 pg/μL to 500 pg/μL, demonstrated 15% to 25% agreement for most PAH components for all calibration curves. For all PAHs the measured concentration was less than the NIST certified concentration. Two possible explanations for this are: 1) commercially prepared PAH standards can differ by 5

to 15% [23], and 2) High Performance Liquid Chromatography (HPLC) analysis was used for certification of 1647d, while GC/MS was used for this study, and previous comparisons for many PAHs have indicated a 12 to 15% lower concentration for GC/MS analysis than for HPLC analysis [24]. By contrast, determined concentrations for the hopane species showed poor agreement. Because poorer QC agreement was realized for the hopane materials, greater uncertainty was associated with the hopane concentration measurements.

Discussion

Table 9 summarizes method performance for each target analyte. The most striking trend in Table 9 is the consistently poorer performance for hopanes as a class in comparison to the PAHs. Comparable calibration verification data and matrix spike recoveries were observed between the two classes, but without exception all of the hopanes could be characterized as exhibiting poorer performance for collocated precision, method detection limits, blank levels, and quality control than all of the PAHs. For example, if the generally most abundant compounds from each class, benzo[g,h,i]perylene and 17α(H),21β(H)-30-norhopane are directly compared, 17α(H),21β(H)-30-norhopane has 50% more variability associated with its collocated precision, a more than 10 times higher MDL, twice the percent difference between measured and assigned certified reference material values, and it is the only one of the two with a measurable blank.

There are several likely reasons for poorer hopane performance. For example, higher detection limits due to greater molecular fragmentation result in a weaker quantitation ion (m/z = 191, see Table 1) for the hopanes. Higher hopane blank levels were not anticipated, and the lack of PAHs in the trip and field blanks suggest low levels of contamination by fuel or machining oil

or by a combustion source low in high molecular weight PAHs, such as diesel exhaust during transport, handling, or exposure in the field. Detectable hopane blanks were limited to a few field and trip blanks, and were never observed in laboratory method blanks that had not been shipped to the field. Collocated precision, though only slightly poorer for hopanes than PAHs, could be explained by inherently greater uncertainty associated with concentrations closer to detection limits than for the PAH concentrations. The variable low level hopane contamination observed in blanks could also have contributed to poorer hopane precision.

Poor quality control for hopanes is a serious concern, especially considering that hopanes are frequently used as an organic tracer for motor vehicle exhaust. PAHs have been extensively studied in a variety of matrices. For the PAHs, validated methods have been published, a number of certified reference materials have been prepared, a variety of alternative standard solutions are commercially available, and numerous laboratories have extensive experience with PAH analysis. In contrast there are few choices for hopane standards, and poorer quality control for hopanes was observed even though the hopane bulk material for the NIST SRM preparation was obtained from the same vendor producing the calibration solution standard. Improvement of hopane quality control is important for establishing that hopane concentration data can be generated comparable in quality to PAH concentration data. The addition of alternative sources of hopane calibration standards from other vendors would help this effort.

These results raise important considerations for PM organic speciation method performance and data quality. While poorer method performance might be expected for highly polar species like organic acids and levoglucosan that generally require derivatization for analysis, or for n-alkanes subject to ubiquitous hydrocarbon interference, these results indicate that even two easily chromatographable hydrocarbons of relatively similar classes, generally

unaffected by significant interference, can exhibit substantial differences in performance. Consequently, even after the development of certified reference materials for the hopane species and implementing laboratory and sample handling procedures to reduce hopane contamination, equivalent data quality for the PAHs and hopanes could not be demonstrated.

In spite of significant method performance differences between the PAHs and hopanes, the results provide both a clearer understanding of PM organic speciation data quality for a typical GC/MS method and a thorough demonstration of high quality data for PM organic speciation measurements. The capability of maintaining calibration consistency for a large number of samples without the need for recalibration for each target analyte was demonstrated. Recoveries of 75% to 85%, consistent with previous estimates for similar methods, have been directly measured for all but one target analyte and consistency between several approaches to determining recovery was demonstrated. Collocated precisions of 20% to 25% that are typically assumed for PM organic components were directly measured for all target analytes. Method detection limits of generally less than 1 pg/m^3 for most PAHs and less than 5 pg/m^3 for 2 of 3 hopanes demonstrate that this is a highly flexible method viable for low volume samples, high time resolution sampling, and very clean environments. Quality control results for PAHs demonstrate that excellent agreement with control solutions is possible with appropriate calibration solution standards and well established SRMs. Overall, good data quality was generally demonstrated for the sampling and analysis of PAHs and hopanes in airborne particulate matter.

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Table 1
Target analytes, surrogates, internal standards, and associated mass ions.

Target/Surrogate	Target or Surrogate	Quantitation & Qualifier Ions[1]	Internal Standard	Quantitation & Qualifier Ions[1]
Pyrene	Target	202, 203	Benz[a]anthracene-d12	240, 241
Chrysene + Triphenylene	Target	228, 229	Benz[a]anthracene-d12	240, 241
3-Methyl Chrysene	Target	242, 243	Benz[a]anthracene-d12	240, 241
Chrysene-d12	Surrogate	240, 241	Benz[a]anthracene-d12	240, 241
Benzo[b+j]fluoranthene	Target	252, 253	Benzo[e]pyrene-d12	264, 265
Benzo[k]fluoranthene	Target	252, 253	Benzo[e]pyrene-d12	264, 265
Benzo[e]pyrene	Target	252, 253	Benzo[e]pyrene-d12	264, 265
Benzo[a]pyrene	Target	252, 253	Benzo[e]pyrene-d12	264, 265
Perylene	Target	252, 253	Benzo[e]pyrene-d12	264, 265
Benzo[b]fluoranthene-d12	Surrogate	264, 265	Benzo[e]pyrene-d12	264, 265
Dibenz[a,h+a,c]anthracene	Target	278, 279	Dibenz[a,h]anthracene-d14	292, 293
Picene	Target	278, 279	Dibenz[a,h]anthracene-d14	292, 293
Indeno[1,2,3-cd]pyrene	Target	276, 277	Dibenz[a,h]anthracene-d14	292, 293
Benzo[g,h,i]perylene	Target	276, 277	Dibenz[a,h]anthracene-d14	292, 293
Indeno[1,2,3-cd]pyrene-d12	Surrogate	288, 289	Dibenz[a,h]anthracene-d14	292, 293
17α(H),21β(H)-30-Norhopane	Target	191, 95	n-Dotriacontane-d66	66, 82
17α(H),21β(H)-Hopane	Target	191, 95	n-Dotriacontane-d66	66, 82
17α(H),21β(H)-22S-Homohopane	Target	191, 95	n-Dotriacontane-d66	66, 82
n-Triacontane-d62	Surrogate	66, 82	n-Dotriacontane-d66	66, 82

[1] Nominal mass ion

Table 2
Median and average ambient concentration ranges for DEARS season 2 samples (n=40 samples).

Target	Median (pg/m^3)[1]	Mean (pg/m^3)[1]	Range (pg/m^3)[1]
Pyrene	94.3	138	47.0 – 493
Chrysene + Triphenylene	120	165	52.5 – 517
3-Methyl Chrysene	33	47	6.5 – 200
Benzo[b+j]fluoranthene	150	190	66.6 - 631
Benzo[k]fluoranthene	71.6	93.3	32.4 - 305
Benzo[e]pyrene	113	142	52.2 - 420
Benzo[a]pyrene	93.5	133	38.4 - 494
Perylene	20.3	300	6.53 – 121
Dibenz[a,h+a,c]anthracene	16.4	22.3	6.35 – 66.3
Picene	10.2	14.8	4.10 – 47.8
Indeno[1,2,3-cd]pyrene	119	153	55.7 - 467
Benzo[g,h,i]perylene	165	202	72.0 - 622
17α(H),21β(H)-30-Norhopane	140	150	39 - 380
17α(H),21β(H)-Hopane	93	110	29 – 270
17α(H),21β(H)-22S-Homohopane	130	150	35 - 420

[1] Excludes analytical precision duplicates and collocated samples

Table 3
Calibration solution verification and precision (calibration stability evaluation)

Calibration Curve	1, 2, 5, 10, 20 pg/uL						10, 20, 50, 100, 200 pg/uL						50, 100, 200, 500 pg/uL					
Prepared Calibration Verification	2 pg/uL (nominal)			10 pg/uL (nominal)			20 pg/uL (nominal)			100 pg/uL (nominal)			100 pg/uL (nominal)			200 pg/uL (nominal)		
n replicates	26			17			24			21			14			9		
Target	**Avg.**	**%RSD[1]**	**%Diff.[2]**	**Avg.**	**%RSD[1]**	**%Diff.[2]**	**Avg.**	**%RSD[1]**	**%Diff.[2]**	**Avg.**	**%RSD[1]**	**%Diff.[2]**	**Avg.**	**%RSD[1]**	**%Diff.[2]**	**Avg.**	**%RSD[1]**	**%Diff.[2]**
Pyrene	2.10	4.7	4.8	10.1	2	1	20.2	2	1	101	2	1	102	2	2	202	2	0.9
Chrysene	2.05	4	2	9.9	2	0.8	20.7	2	3	98	1	2	99	0.8	1	199	1	0.9
3-Methyl Chrysene	1.9	8	5	9.5	4	5	20.5	4	3	90.3	2.6	9.5	93.7	2.5	6.1	186	3	6.6
Benzo[b]fluoranthene	2.07	4.7	3	10.1	3	0	21.2	2	5.2	95.8	2.2	4.9	98	2	2	196	2	3
Benzo[k]fluoranthene	1.90	6.3	5.6	9.6	4	5	20.5	3	2	90.0	3.2	10.4	93.8	3.2	6.7	190	3	5.6
Benzo[e]pyrene	2.05	4.6	2	10.2	3	2	21.2	2	5.8	97.7	1.2	2.4	100	1	0	199	0.7	0.5
Benzo[a]pyrene	2.00	6.8	0.3	9.8	6	2	21.5	5.0	7.1	91.9	3.3	8.3	96.0	2.6	4.2	194	2	3
Perylene	1.86	8.9	6.9	9.6	5	4	21.0	4	4.8	87.2	3.4	12.8	92.3	3.3	7.7	183	4	8.4
Dibenz[a,h]anthracene	2.00	5.2	0.1	10.1	4	0.9	20.9	2	5.0	95.1	1.5	4.6	97.2	1.1	2.6	194	1	3
Picene	2.10	7.0	4	10.8	6	8	22.0	4	9.5	98	2	3	101	2	0	199	1	1
Indeno[1,2,3-cd]pyrene	2.03	5	1	10.2	3	2	21.6	2	7.6	96.9	1.3	3.4	99	1	1	198	1	1
Benzo[g,h,i]perylene	1.98	4	1	10.0	2	1	20.2	2	0	99	1	2	99	1	1	199	0.7	1
17α,21β-30-Norhopane	*	*	*	*	*	*	22	3	10	100	4	3	100	4	4	210	4	4
17α,21β-Hopane	*	*	*	*	*	*	22	3	9	99	3	1	100	3	1	200	3	0.5
17α,21β-22S-Homohopane	*	*	*	*	*	*	21	6	3	100	4	0	100	4	0	200	3	1

[1] %RSD: relative standard deviation of average calibration verification solution concentration for n replicates
[2] %Difference: difference between average concentration for n replicates and prepared calibration verification solution
* hopanes are below limits of quantitation at this level (see Table 7).

Table 4
Percent recovery: mean % for n replicates ± % RSD.

Target	Initial Method Proficiency n=9	Batch Matrix Spikes n=8	Deuterium-Labeled Surrogates[1] n=22
Pyrene	71.4 ± 8.8%	63.9 ± 14.0%	
Chrysene	77.4 ± 5.0%	78.0 ± 14.7%	76.3 ± 5.9%[2]
3-Methyl Chrysene	81 ± 10%	84.6 ± 13.0%	
Benzo[b]fluoranthene	80.4 ± 6.8%	82.8 ± 16.0%	85.2 ± 10.0%[3]
Benzo[k]fluoranthene	79.5 ± 6.7%	85.4 ± 15.6%	
Benzo[e]pyrene	79.7 ± 5.8%	84.2 ± 16.5%	
Benzo[a]pyrene	79.4 ± 7.7%	86.0 ± 17.4%	
Perylene	75.1 ± 14.0%	80.5 ± 18.5%	
Dibenz[a,h]anthracene	81.0 ± 6.0%	80.5 ±14.4%	
Picene	82.3 ± 8.7%	85.3 ± 13.5%	
Indeno[1,2,3-cd]pyrene	86.5 ± 6.7%	79.5 ± 15.6%	82.6 ± 9.8%[4]
Benzo[g,h,i]perylene	85.2 ± 6.2%	80.0 ± 15.1%	
17α(H),21β(H)-30-Norhopane	76 ± 9%	79 ± 23	
17α(H),21β(H)-Hopane	74 ± 8%	78 ± 25%	
17α(H),21β(H)-22S-Homohopane	77 ± 9%	78 ± 26%	
n-Triacontane-d62			83.2 ± 12.2%

[1] Surrogate recoveries determined from spiked filter samples
[2] Measured recovery for d12 analog of chrysene
[3] Measured recovery for d12 analog of benzo[b]fluoranthene
[4] Measured recovery for d12 analog of indeno[1,2,3-cd]pyrene

Table 5
Analytical precision, collocated sample precision, and total percent propagated error

Target	Analytical Duplicate Precision[1] n=10	Collocated Sampling Precision[1] n=5	Total Percent Propagated Error n=40
Pyrene	3%	22%	15%
Chrysene + Triphenylene	3%	18%	16%
3-Methyl Chrysene	5%	17%	15%
Benzo[b+j]fluoranthene	7%	19%	19%
Benzo[k]fluoranthene	2%	15.4%	20%
Benzo[e]pyrene	0.6%	16%	18%
Benzo[a]pyrene	1%	17%	23%
Perylene	2%	20%	20%
Dibenz[a,h+a,c]anthracene	2%	15%	15%
Picene	5%	22%	15%
Indeno[1,2,3-cd]pyrene	2%	15%	17%
Benzo[g,h,i]perylene	1%	15%	16%
17α(H),21β(H)-30-Norhopane	5%	22%	27%
17α(H),21β(H)-Hopane	8%	22%	28%
17α(H),21β(H)-22S-Homohopane	7%	25%	27%

[1] Calculated using Equation 3 described in Precision section.

Table 6
Method Detection Limits and Limits of Quantitation.

Target	MDL[1] pg/μL	LOQ[2] pg/μL	MDL[1] pg/m^3	LOQ[2] pg/m^3	%Samples >LOQ
Pyrene	0.38	1.3	0.59	2.1	100%
Chrysene	0.29	1.0	0.48	1.6	100%
3-Methyl Chrysene	0.49	1.7	0.83	2.9	100%
Benzo[b]fluoranthene	0.29	0.99	0.43	1.5	100%
Benzo[k]fluoranthene	0.38	1.3	0.67	2.3	100%
Benzo[e]pyrene	0.36	1.2	0.46	1.6	100%
Benzo[a]pyrene	0.42	1.1	0.81	2.2	100%
Perylene	1.2	1.7	2.9	4.1	100%
Dibenz[a,h]anthracene	0.31	1.1	0.41	1.4	100%
Picene	0.41	1.4	0.81	2.8	100%
Indeno[1,2,3-cd]pyrene	0.42	1.4	0.81	2.7	100%
Benzo[g,h,i]perylene	0.31	1.1	0.37	1.3	100%
17α(H),21β(H)-30-Norhopane	1.6	5.4	4.5	15	100%
17α(H),21β(H)-Hopane	1.7	5.8	2.4	8.3	100%
17α(H),21β(H)-22S-Homohopane	2.9	9.9	11	37	98%

[1]MDL = Method Detection Limit
[2]LOQ = Limit of Quantitation

Table 7
Limits of Detection and Quantitation and Average Method, Trip, and Field Blank Levels (Mean ± Standard Deviation).

Target	MDL[1] pg/m^3 equiv^3	LOQ[2] pg/m^3 equiv^3	Method Blank pg/m^3 equiv^3 (n=10)	Trip Blank pg/m^3 equiv^3 (n=5)	Field Blank pg/m^3 equiv^3 (n=5)	Percent Avg. Trip Blank per Avg. Sample
Pyrene	0.59	2.1	0.1 ± 0.2	0.7± 0.4	0.7 ± 0.3	0.5%
Chrysene + Triphenylene	0.48	1.6	*	0.35 ± 0.07	0.63 ± 0.19	0.21%
Benzo[b+j]fluoranthene	0.43	1.5	*	0.2 ± 0.1	0.20 ± 0.04	0.1%
17α(H),21β(H)-30-Norhopane	4.5	15	*	12 ± 4	12 ± 3	8.0%
17α(H),21β(H)-Hopane	2.4	8.3	*	11 ± 1	*	10%

*Not Detected
[1]MDL = Method Detection Limit
[2]LOQ = Limit of Quantitation
[3] Based on collected air volume

Table 8
Independent Quality Control evaluation: comparison of prepared and measured values, (percent difference of measured from prepared concentration).

Target	Source	Prepared[1] (pg/μL)	Measured (pg/μL)	Difference %
Pyrene	NIST - SRM 1647d	169	155	8.3%
Chrysene	NIST - SRM 1647d	73.4	61.6	16.1%
3-Methyl Chrysene	Chiron	80	71	10%
Benzo[b]fluoranthene	NIST - SRM 1647d	83.4	67.6	18.9%
Benzo[k]fluoranthene	NIST - SRM 1647d	94.4	71.4	24.4%
Benzo[e]pyrene	AccuStandard	100.0	93.6	6.4%
Benzo[a]pyrene	NIST - SRM 1647d	98.2	75.7	22.9%
Perylene	Absolute Standards	100.4	89.7	10.7%
Dibenz[a,h]anthracene	NIST - SRM 1647d	70.8	60.8	14.1%
Picene	Absolute Standards	102	77.4	25%
Indeno[1,2,3-cd]pyrene	NIST - SRM 1647d	85.6	74.7	12.7%
Benzo[g,h,i]perylene	NIST - SRM 1647d	73.6	65.6	11%
17α(H),21β(H)-30-Norhopane	NIST - SRM 2266	90	110	22%
17α(H),21β(H)-Hopane	NIST - SRM 2266	117	79	32%
17α(H),21β(H)-22S-Homohopane	NIST - SRM 2266	106	160	51%

[1] Prepared from certified reference materials.

Table 9
Summary of key method performance parameters.

Target[1]	Matrix Spike Recovery n=8	Collocated Sampling Precision n=5	Method Detection Limit (pg/m^3)	Trip Blank (% of Avg Sample)	Quality Control[2] (~100 pg/µL)
Pyrene	63.9±14.0%	22%	0.59	0.5%	8.3%
Chrysene + Triphenylene	78.0±14.7%	18%	0.48	0.21%	16.1%
3-Methyl Chrysene	84.6±13.0%	17%	0.83	*	10%
Benzo[b+j]fluoranthene	82.8±16.0%	19%	0.43	0.1%	18.9%
Benzo[k]fluoranthene	85.4±15.6%	15.4%	0.67	*	24.4%
Benzo[e]pyrene	84.2±16.5%	16%	0.46	*	6.4%
Benzo[a]pyrene	86.0±17.4%	17%	0.81	*	22.9%
Perylene	80.5±18.5%	20%	2.9	*	10.7%
Dibenz[a,h+a,c]anthracene	80.5±14.4%	15%	0.41	*	14.1%
Picene	85.3±13.5%	22%	0.81	*	25%
Indeno[1,2,3-cd]pyrene	79.5±15.6%	15%	0.81	*	12.7%
Benzo[g,h,i]perylene	80.0±15.1%	15%	0.37	*	11%
17α(H),21β(H)-30-Norhopane	79 ±23%	22%	4.5	8.0%	22%
17α(H),21β(H)-Hopane	78 ±25%	22%	2.4	10%	32%
17α(H),21β(H)-22S-Homohopane	78 ±26%	25%	11	*	51%

[1] Isomers shown to reflect ambient data
[2] Percent difference of measured from prepared concentration
* Not Detected