

Interlaboratory comparison of HPLC-fluorescence detection and GC/MS: analysis of PAH compounds present in diesel exhaust

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Abstract

For laboratories involved in polycyclic aromatic hydrocarbon (PAH) analyses in environmental samples, it is very useful to participate in interlaboratory comparison studies which provide a mechanism for comparing analytical methods. This is particularly important when PAH analyses are routinely done using a single technique. The results are reported for such an interlaboratory comparison study, in which the four selected participating laboratories quantitatively analyzed several PAH compounds in diesel exhaust samples. The samples included particle and vapor phase extracts collected and prepared at Michigan Technological University (MTU PE and MTU VE, respectively), a diesel particle extract prepared by the National Institute for Standards and Technology (NIST, SRM 1975), and a fully characterized diesel particle sample (NIST SRM 1650). One of the laboratories used only HPLC-FLD, one used only GC/MS and two laboratories used both methods for the routine analysis of PAH in environmental samples.

Data were obtained for five PAH compounds: fluoranthene, pyrene, benz[*a*]anthracene, benzo[*a*]pyrene, and benzo[*g,h,i*]perylene. The mean PAH levels found for SRM 1650 were outside the range reported by NIST. The range in the reported means was from 24% lower than certified for benz[*a*]anthracene to 41% higher for benzo[*g,h,i*]perylene.

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For the previously uncharacterized samples in this study (SRM 1975, MTU PE and MTU VE), two-thirds of the reported results were higher for the HPLC-FLD method than for the GC/MS. The range in differences between methods was from -54 to + 31% calculated as the difference in GC/MS value relative to the HPLC value for each of the compared compounds.

Coefficients of variation for the uncharacterized samples appeared to be higher, in most (but not all) cases, for the HPLC-FLD than for the GC/MS. The resolution of certain PAH isomers (e.g. benz[*a*]anthracene and chrysene, or the benzofluoranthenes), was better, as expected, for HPLC than for GC. Generally lower detection limits (by an order of magnitude or more) were reported for GC/MS than for HPLC-FLD. On the basis of this limited study, it seems as though significant differences may exist between laboratories, if not between methods, in the analysis of certain PAH compounds in real diesel samples by HPLC-FLD compared to GC/MS. If possible, measurements should be made using both methods. This is particularly important where potential interferences are undefined or subject to change, as is frequently the case with real environmental samples. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Polycyclic aromatic hydrocarbons; Diesel exhaust emissions; Interlaboratory comparison; Analysis

1. Introduction

The particulate and gaseous phase emissions generated by the incomplete combustion of diesel fuel and lubricating oil have been extensively studied over the past three decades (for reviews, see Johnson et al. [1] and Levson [2]). Both the International Agency for Research on Cancer [3] and the US Environmental Protection Agency (EPA, [4]) have classified diesel exhaust as a probable human carcinogen. In addition, the National Institute for Occupational Safety and Health has declared diesel exhaust to be a potential occupational carcinogen [5].

Among the numerous organic components of diesel exhaust are polycyclic aromatic hydrocarbons (PAH), some of which are known rodent (and suspected human) carcinogens, e.g. benz[*a*]anthracene, chrysene, benzo[*b*, *j* and *k*]fluoranthenes, and benzo[*a*]pyrene or cocarcinogens, e.g. fluoranthene and pyrene. For the registration of fuels and fuel additives the US Environmental Protection Agency requires engine emissions measurements that include the quantification of certain specified PAH in both particulate and vapor phase emissions [6]. Therefore, although the emissions of specific PAH are not currently regulated, there is considerable interest in their accurate measurement.

A number of analytical methods have been developed and applied to the quantification of PAH. Foremost among these are gas chromatography/mass spectrometry (GC/MS), and high performance liquid chromatography (HPLC) with fluorescence detection. Both of these methods have adequate resolution and sensitivity to be used for the quantification of trace quantities of PAH in complex environmental matrices [7]. It is qualitatively advantageous to employ both methods whenever possible. Standard reference materials SRM 1580 (shale oil), 1582 (petroleum crude oil), 1597 (coal tar extract), 1649 (urban dust) and 1650 (diesel particulate matter) were all certified by the National Institute for Standards and Technology (NIST) for levels of certain PAH using both GC/MS and HPLC/fluorescence, which were found to give statistically equivalent results [8–10]. EPA method 610 also specifies reverse phase HPLC with fluorescence

Table 1
Summary of samples analyzed for the interlaboratory comparison

Sample	Description
NIST SRM 1650	Characterized diesel particulate matter (solid particles, extracted by participants)
NIST SRM 1975	Investigational SRM, dichloromethane extract of diesel particulate matter
MTU PE	Dichloromethane extract of diesel exhaust particles generated at MTU
MTU VE	Dichloromethane extract of diesel exhaust vapor phase generated at MTU

detection for the quantification of PAH in municipal and industrial discharges, with additional evidence for confirmation obtained using GC/MS [11].

For laboratories involved in environmental PAH analyses, it is very useful to participate in interlaboratory quality assurance testing programs which provide a mechanism for comparing analytical methods using real samples. This is particularly important when PAH analyses are routinely done using a single method. The results of an interlaboratory comparison study are reported here, in which the selected participants quantitatively analyzed by their routinely used methods certain specific PAH compounds in diesel exhaust samples. All of the participants had previous experience in the analysis of diesel exhaust samples and had published results of PAH studies prior to conducting the work reported here.

The PAH compounds selected for analysis were fluoranthene (FLA), pyrene (PYR), benz[a]anthracene (BaA), benzo[*a*]pyrene (BaP), and benzo[*g,h,i*]perylene (BP). These compounds are of interest due to their suspected health effects and have certified levels in the diesel particulate matter standard reference material (SRM 1650).

The samples analyzed in this study included particulate and vapor phase diesel exhaust samples collected at MTU as well as a diesel particle extract prepared by NIST (SRM 1975) and a fully characterized particulate sample (NIST SRM 1650). SRM 1650 was available as a certified standard reference material prior to the start of this study, whereas SRM 1975 was not, and was included in the sample matrix to provide an indication of the precision and, for certified components, the accuracy of the analytical method used. This sample (SRM 1650) was also intended to provide an indicator of sample integrity during shipping, and to serve as a benchmark when interpreting results which appear to be method-dependent. Table 1 gives a summary description of the four test samples.

2. Methods

2.1. Sample preparation

The MTU extracts were prepared from diesel particles and vapor phase exhaust material collected in the dynamometer test cell at MTU [12,13]. The engine used in this study was a 1988 Cummins LTA-10 300, a heavy-duty six-cylinder direct injection diesel engine. The engine was turbocharged and after-cooled and rated at 224 kW at 1900 rpm. The fuel was a commercial (low-sulfur) diesel fuel typical of that used for

on-highway trucks in the United States. The engine was operated at EPA steady-state mode 9 (75% load at rated speed) without any after-treatment emissions control devices. The exhaust samples were collected (after a 2-h warm-up) from a partial-flow dilution tunnel, where the sampling temperature was held constant ($45 \pm 1^\circ\text{C}$) at a dilution ratio of approximately 13:1.

Particulate and vapor phase samples were collected on Teflon-coated glass fiber filters (508×508 mm, Pallflex, Putnam, CT) and XAD-2 resin (40 g, pre-extracted with dichloromethane, Supelco, Bellefonte, PA), respectively, using a high-volume sampler. Before exposure, the filters were equilibrated at $45 \pm 1\%$ relative humidity at $23.5 \pm 1^\circ\text{C}$ for 24 h and then weighed. Following exposure, they were ammoniated, re-equilibrated and reweighed. The exposed filters and XAD-2 were extracted using a Soxhlet apparatus and dichloromethane (180 ml for the filters and 350 ml for the XAD-2 samples). The extracts were mixed with anhydrous sodium sulfate (ca. 1 g), filtered, and reduced in volume using a rotary evaporator. All of the filter extracts were pooled and diluted to a final volume of 50.0 ml with dichloromethane. Likewise, all of the XAD-2 extracts were pooled and diluted to 50.0 ml. The masses of the extracts (and concentrations of the solutions) were then determined by taking 100 μl samples of the pooled extracts to dryness on pre-tared glass fiber filter disks and subsequently reweighing them.

Portions (1.0 ml) of the particulate (MTU-PE) and vapor phase extracts (MTU-VE) were transferred to individual amber glass HPLC vials with Teflon-lined silicone septum screw caps and stored in the freezer ($-15 \pm 5^\circ\text{C}$) until shipment on dry ice, along with the samples from NIST. All of the participants received the samples in good condition with dry ice still remaining in the packages. The laboratories were provided with the information given in Table 1, as well as the densities for liquid samples, prior to analysis.

2.2. PAH quantification methods

Table 2 gives a summary of the analytical methods used by the participants. It should be noted that although two of the participants routinely used both analytical methods, for

Table 2
Summary of analytical methods used by participants^a

Laboratory	Extraction method	Pre-analytical clean-up	Analytical method (s)
A	Soxhlet, DCM ^b	SPE: Florisil, C ₁₈	RPHPLC/FLD
B	Soxhlet, DCM ^c	LC: silica gel	GC/MSD, HRGC/HRMS
C	Boiling, Toluene	HPLC: Sephadex	GC/MS, RPHPLC/FLD
D	Soxhlet, DCM ^d	SPE: silica Sep-pak SPE: aminopropylsilane	GC/MS RPHPLC/FLD

^aAbbreviations: DCM = dichloromethane, FLD = fluorescence detection, LC = liquid chromatography, HPLC = high performance LC, RPHPLC = reverse phase HPLC, GC = gas chromatography, HRGC = high-resolution GC, MS = mass spectrometry, HRMS = high resolution mass spectrometry SPE = solid-phase extraction.

^b24 h extraction time.

^c16–20 h extraction time.

^dNormal method used by this lab; however extraction only required in present study for NIST SRM sample, for which there were no results reported from lab D.

Table 3
Summary of test results for the characterized sample, NIST SRM 1650

Compound	Certified mean ^a (ug/g)	Reported mean ^b (ug/g)	Standard deviation ^c	Deviations from the mean ^d			
				A _h	B _g	C _g	C _h
FLA	51 ± 4	57.4	13.8	1.1	-1.2	-0.27	0.41
PYR	48 ± 4	42.7	4.09	1.1	-1.1	0.53	-0.49
BaA	6.5 ± 1.1	4.95	0.699	-1.1	0.56	0.59	n/a ^e
BaP	1.2 ± 0.3	1.51	0.953	-1.1	-0.54	0.97	0.70
BP	2.4 ± 0.6	3.38	1.67	-0.47	0.55	-1.2	1.1

^aMean certified by NIST to represent concurrence of two methods, mean ± 2σ.

^bMean of the three labs reporting results, *n* = 4 in all cases (except BaA) due to one lab reporting two values using two methods.

^cStandard deviation calculated from all reported values except statistical outliers.

^dNumber of standard deviations from reported mean, A, B, and C denote laboratories; subscripts *g* and *h* denote GC/MS and HPLC-FLD, respectively..

^en/a = not applicable; value reported for this lab was a statistical outlier.

some of the samples only one method was employed. The test matrix therefore ended up unbalanced from the viewpoint of statistical analysis.

2.3. Statistical methods

The data set obtained from this study was rather small and unbalanced; results were therefore not subjected to rigorous statistical analysis. All of the data are presented as reported by the participants, except for several outliers which were deleted based on the *Q*-test [14]. The means, standard deviations and coefficients of variation (CV) are given for each compound by laboratory and by method. In the summary tables, the data are also represented as the number of standard deviations they are from the calculated mean.

3. Results

The results are summarized in Tables 3–8. The laboratories have been coded as Ah, Bg, Cg, Ch, Dg, and Dh, where each upper case letter represents one of the participating

Table 4
Comparison of HPLC and GC/MS results for NIST SRM 1650

Compound	HPLC ^a	GC/MS ^a	% Difference ^b
	mean (CV)	mean (CV)	
FLA	67.8 (10)	47.0 (20)	-31
PYR	44.0 (10)	41.5 (12)	-5.7
BaA	4.14 (n/a) ^c	5.35 (0.26)	+29
BaP	1.31 (94)	1.72 (59)	+31
BP	3.88 (47)	2.87 (70)	-26

^aNumber of labs reporting = 2, unless noted.

^b% difference = ((GC/MS-HPLC)/HPLC) × 100.

^cn/a, not applicable, *n* = 1.

Table 5
Summary of test results for SRM 1975, diesel particulate matter extract

Compound	Draft certified mean (ug/g)	Mean ^a (ug/g)	Standard deviation ^a	Deviations from the mean ^b				
				A _h	B _g	C _h	D _h	D _g
FLA	916	758	152	−1.4	0.15	−0.47	0.73	1.1
PYR	26.7	24.3	9.40	0.49	−0.56	−1.1	1.5	−0.36
BaA	5.36	7.60 ^c	6.37	−0.60	−0.54	1.5	n/r ^d	−0.35
BaP	n/r	1.03	0.68	−0.90	−0.30	−0.23	1.4	n/a ^e
BP	2.61	2.57 ^c	1.11	0.034	−1.3	1.1	n/r	0.19

^aMeans and standard deviations calculated from all reported values except statistical outliers; $n = 5$ unless noted.

^bA, B, C and D denote laboratories; subscripts g and h denote GC/MS and HPLC-FLD, respectively.

^c $n = 4$.

^dn/r = not reported.

^en/a = not applicable, level reported as < MDL.

Table 6
Summary of test results for the uncharacterized samples, MTU PE and MTU VE

Compound	Mean ($\mu\text{g/g}$) ^a	Standard deviation ^a	Coefficient of variation (%)	Number of deviations from the mean ^b			
				A _h	B _g	D _h	D _g
<i>(A) MTU PE</i>							
FLA	191	56.4	30	1.4	−1.0	0.035	−0.35
PYR	271	53.2	20	1.0	−1.1	0.66	−0.62
BaA	44.0 ^c	15.4	35	−1.3	−0.18	1.0	0.43
BaP	13.4	3.77	28	−1.4	0.064	0.89	0.46
BP	5.75 ^d	1.16	20	n/a ^e	−1.1	0.85	0.25
<i>(B) MTU VE</i>							
FLA	54.3 ^c	5.36	9.9	n/i ^f	0.49	0.66	−1.2
PYR	56.6	21.8	38	1.2	0.36	−1.2	−0.40

^a Means and standard deviations calculated from all reported values except statistical outliers; $n = 4$ unless noted.

^b A, B, C and D denote participating laboratories; subscripts g and h denote GC/MS and HPLC-FLD, respectively.

^c $n = 3$.

^d $n = 2$.

^e n/a = not applicable, value below detection limit.

^f n/i = not included, statistical outlier.

laboratories and the subscripts g and h refer to GC/MS and HPLC/FLD, respectively, and designate the analytical method that was used by that laboratory. Tables 3 and 4 provide results for NIST SRM 1650 (three of the four labs reported concentrations of PAH for this sample) and Tables 5–8 provide the results for NIST SRM 1975, MTU-PE, and MTU-VE samples. All four laboratories reported results for most of the specified PAH compounds in SRM 1975, and the PE and VE extracts. The data reported by Lab C_h for the PE sample were deleted as outliers, as was the BaA level reported by Lab C_h for SRM 1650 and the FLA level reported by Lab A_h for the MTU VE sample.

Table 7
Comparison of HPLC and GC/MS results for NIST SRM 1975

Compound	Mean draft certified	HPLC ^a mean (CV)	GC/MS ^b mean (CV)	% Difference ^c
FLA	916	697 (24)	849 (11)	+22
PYR	26.7	27.2 (44)	20.1 (6.7)	−26
BaA	5.36	10.4 (90)	4.77 (17)	−54
BaP	n/r ^d	1.10 (74)	0.826 (n/a) ^e	−25
BP	n/r	3.21 (26)	1.94 (61)	−40

^a $n = 3$ labs reporting.

^b $n = 2$ labs reporting, unless noted.

^c % difference = ((GC/MS-HPLC)/HPLC) × 100.

^d n/r = draft certified mean not reported by NIST for this compound.

^e n/a, not applicable, $n = 1$.

Table 8
Comparison of HPLC and GC/MS results for MTU particulate extract (PE)

Compound	HPLC ^a mean (CV)	GC/MS ^a mean (CV)	% Difference ^b
FLA	219 (31)	163 (26)	-26
PYR	282 (22)	260 (25)	-7.8
BaA	37.3 (50)	50.6 (26)	+36
BaP	11.6 (43)	15.2 (14)	+31
BP	6.05 (n/a) ^c	5.61 (29)	-7.3

^a $n = 2$ labs reporting, unless noted.

^b % difference = ((GC/MS-HPLC)/HPLC) \times 100.

^c n/a, not applicable, $n = 1$.

The results can be discussed as two distinct groups of data. The first group includes the participants' results for SRM 1650. The identity of this sample was known by the participants and since there are known (and published) levels of specific PAH compounds in this sample, it was not a true unknown, but rather a control sample. The remaining three samples, which comprise the second group, were true unknowns.

For SRM 1650, the means of the reported values (Table 3) in every case fell outside the range reported by NIST which is defined as the average values obtained from at least two independent analytical techniques and the uncertainties which are two times the standard deviations for the average values (SRM 1650 Certificate of Analysis). The reported mean was higher than the range for three compounds and lower in the other two cases. The range in the reported means (all data) compared to the certified means was from 24% lower than certified for BaA to 41% higher than certified for BP. The results for SRM 1650 have also been presented in terms of how far they are from the mean, i.e. as the number of standard deviations they are from the reported mean (Table 3).

A summary of the means and CV for each compound by method and the percent-difference between methods is given in Table 4 for SRM 1650. The differences between methods were from 5.6% to 31%. For three of the five compounds; the HPLC method gave higher means than the GC/MS method.

A mean, standard deviation, and CV calculated from the reported values is given in Table 5 for each compound for SRM 1975 and in Table 6 for the MTU PE and VE samples. The participants' results have also been presented as a number of standard deviations from the mean. The means and CV by method and the differences between methods are given for SRM 1975 (Table 7) and for the MTU PE sample (Table 8). These data indicate a method bias for the uncharacterized samples, but due to the small number of participants, the method bias may not be statistically significant. For four of the five compounds listed for SRM 1975 (Table 7), the reported means were higher (by 25% or more) for HPLC than for GC/MS. The CV were also generally higher for the HPLC method, although it is important to note that there were an unequal number of results for SRM 1975, i.e. $n = 2$ for GC/MS and $n = 3$ for HPLC.

Although SRM 1975 had a non-characteristic FLA/PYR ratio (ca. 35:1), there was relatively good agreement among the participants on the identity and quantities of these

two compounds. The largest discrepancy between methods in this study, however, was for BaA in SRM 1975, where the level found by GC/MS was 54% lower than for HPLC. Since the completion of this study, NIST has compiled sufficient data to issue “draft certified levels” of certain PAH in SRM 1975 (Table 5). For FLA and BaA the draft certified values are very close to the mean GC/MS levels, while for PYR, the HPLC mean is much closer to the draft certified level.

For the MTU PE sample, the HPLC means were higher in three of five cases compared to the GC/MS means (Table 8). Where CV values differed between methods, they were higher for the HPLC method.

Since the test matrix turned out to be statistically unbalanced for this study, it was not possible to determine whether or not significant differences existed between data sets. It does appear though, considering the few data points available for both the characterized and uncharacterized samples, that there is a tendency for the HPLC-FLD results to be higher than the GC/MS results. The accuracy of these methods cannot really be assessed by this study, as there is no independent reference method for comparison.

The HPLC-FLD method demonstrates an advantage in resolution of some of the PAH isomers analyzed in this study. It is also generally expected that the sensitivity will be better for this method than for GC/MS; however, that was not the case in this study. There were lower minimum detection limits reported for GC/MS than for HPLC-FLD.

4. Conclusions

(1) The mean PAH levels found for the NIST SRM 1650 diesel particulate matter samples were outside the range reported by nist at the time of the study.

(2) For the previously uncharacterized samples in this study (SRM 1975, MTU PE and MTU VE), two-thirds of the reported results were higher for the HPLC-FLD method than for the GC/MS.

(3) Coefficients of variation for the uncharacterized samples appeared to be higher for the HPLC-FLD than for the GC/MS.

(4) The resolution of certain PAH isomers (e.g. benz[*a*]anthracene and chrysene, or the benzofluoranthenes), was better, as expected, for HPLC than for GC; however, generally lower detection limits were reported for GC/MS than for HPLC-FLD.

5. Recommendations

Based upon the results of this limited study, the following recommendations are made:

(1) Significant differences may exist between laboratories, if not between methods, in the analysis of certain PAH compounds in real diesel samples by HPLC-FLD compared to GC/MS. The value of future comparison studies could be enhanced by including samples for analysis by various techniques using identical preparation methods, or samples prepared by various methods but analyzed using the same technique. A comparison study involving a larger number of laboratories (both neophytes and

experienced laboratories) would be useful in the assessment of variations in analytical methods.

(2) If possible, PAH measurements should be made using both methods. This is particularly important in an experiment where the sample matrix is undefined and subject to change during the experiment, as for example, when measuring PAH emissions from engines while varying the engine conditions.

(3) The differences that do exist between laboratories and methods could also be evaluated and improved by the implementation of an external quality control program which includes improved and defined analysis procedures.

(4) The standard reference materials currently available for PAH analysis reflect early studies on health effects and the EPA list of sixteen priority pollutant PAH. The choice of analytes should be reviewed and expanded, if necessary, in order to provide meaningful materials that are important for the studies of health effects and atmospheric chemistry and reflect the current thinking in these areas.

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