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Validation of a gas chromatography/mass spectrometry method for the quantification of aerosolized Jet Propellant 8

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Abstract

Jet Propellant 8 (JP-8) jet fuel is a kerosene-based fuel containing hundreds of hydrocarbons used by the military in NATO countries. Previous rodent inhalation studies carried out with aerosolized JP-8 never evaluated the exposure chamber atmosphere. For this reason, our laboratory developed an analytical method, with an accuracy of better than 80% and precision of better than 20%, for JP-8 aerosol and vapor samples using gas chromatography/mass spectrometry (GC/MS). A method was developed for quantification of selected individual components of JP-8 and for the total amount of JP-8 in aerosolized fuel. A 34 component surrogate hydrocarbon mixture (SHM) was developed and used for simultaneous analysis of the individual components. Three separate runs containing a standard curve and five replicates each at the selected concentrations were analyzed for both the SHM and neat JP-8. The resulting interday accuracy (100 − percent relative error) and precision (relative standard deviation) values for the SHM were 86.5% or better and 8.0% or better, respectively. The intraday accuracy and precision values ranged from 99.29% to 84.50% and 0.97% to 12.4%, respectively. For the total amount of JP-8 in aerosol and vapor, the interday accuracy was 83.7% or better and interday precision was 7.0% or better. The intraday accuracy and precision values ranged from 94.8% to 80.4% and 2.4% to 10.5%, respectively. We then used this method to analyze samples collected from an inhalation chamber. From the data obtained, we are able to account for approximately 40–44% of the mass of the aerosol portion and 68–70% of the mass of the vapor portion. The aerosol represented 6–10% of the total mass of the aerosolized JP-8 fuel with the remaining portion being the vapor. From these experiments individual components were identified for further in vivo and in vitro toxicological testing.

Keywords: JP-8; GC/MS; Hydrocarbon analysis; Jet fuel; Inhalation studies

1. Introduction

Jet Propellant 8 (JP-8) is a kerosene-based jet fuel used in a variety of military vehicles, in addition to jet engine aircraft with an average molecular weight of 180 Daltons [1,2]. JP-8 is the battlefield fuel for all NATO ground and air forces. This fuel is a complex hydrocarbon mixture containing over four hundred hydrocarbons and is composed primarily of four classes of compounds: *n*-alkanes and isoalkanes, olefins, naphthenes, and aromatics, and there is high variability from one batch of fuel to another. About 18% of this fuel is aromatic hydrocarbons, while the remaining components are aliphatic alkanes and their isomers (9% C_8–C_9, 65% C_{10}–C_{14}, and 7% C_{15}–C_{17}). JP-8 is commer-

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cial jet fuel (Jet-A) with very small amounts of proprietary additives.

The National Occupational Exposure Survey by the National Institute for Occupational Safety and Health estimated that over 1 million employees were exposed to kerosene [3]. Exposure to JP-8 fuel is common for flight line personnel and fuel cell workers. In cold climates aircraft engines using JP-8 experience "cold starts" which results in spraying of non-combusted aerosolized fuel onto personnel in the vicinity of the jet [2]. Few human health studies have been undertaken with fuel exposed workers. In one such study, Scandinavian fuel workers reported fatigue, headache, dizziness, nausea, anxiety, vegetative hyperreactivity, and attention span deficient [4–6]. In another study, JP-8 exposed workers are reported to display subtle deficits in their ability to maintain balance [7].

The published animal toxicity findings for inhaled JP-8 range from relatively minimal for vapors [8–10] at occupationally relevant concentrations to potentially adverse for aerosol and vapor mixture [11–17]. In all cases, the inhalation chamber JP-8 exposures were simply calculated and reported as the sum of all detected hydrocarbons (mass divided by volume of air sampled) using various analytical methods. In the aerosol and vapor exposed animals, the chamber concentrations were based on impactor plate weights, which captured only the aerosol droplets.

In this study we report a GC/MS analytical method, with accuracy of 80% or better and precision of better than 13%, for aerosolized JP-8 sampled directly from an inhalation chamber. The analytical method allowed for the quantification of 34 individual components of aerosolized JP-8 and the determination of the total amount of aerosolized JP-8. The importance of this study is that a validated analytical method has not previously been available to characterize the hydrocarbon composition of aerosolized JP-8. Furthermore, the atmosphere of an animal exposure chamber for aerosolized JP-8 has never been evaluated. This critical information will assist in obtaining a better understanding of the lung toxicity and immunotoxicity findings in rats and mice when exposed to the aerosolized JP-8 [11–17]. Currently, it is unknown if a hydrocarbon fraction or particular components of aerosolized JP-8 are responsible for these toxicities.

2. Experimental

2.1. GC/MS sample analyses

All samples were analyzed with an Agilent 6890 gas chromatograph equipped with an autosampler and a 5973N mass spectrometer operated in full scan mode using a range of 35–270 *m*/*z*. The GC carrier gas was helium, and the ionization mode of the MS was electron ionization. 150–200 μL of each sample at 0 °C was dispensed into a 300 μL vial insert housed in a 2 mL autosampler vial (National Scientific, San Rafael, CA, USA). All sample vials were separated by chloroform blank vials to ensure that no carryover existed in the system. A Petrocol DH 150 (Supelco, Bellefonte, PA, USA) column, with dimensions 150 m × 0.25 mm × 1 μm, was used to provide adequate separation of the hydrocarbons. Samples were introduced as a liquid. The injection volume was 3 μL and the split ratio was 3:1. The inlet temperature was 250 °C, column flow was 1 mL/min (constant flow mode), initial oven temperature was 90 °C, ion source temperature was 230 °C, and the quadrupole temperature was 150 °C. The oven temperature was held at 90 °C for 30 min and then increased to 120 °C using 5 °C/min increments. The temperature was then held at 120 °C for 40 min and then increased to 140 °C using 5 °C/min increments. The oven was held at this temperature for 40 min and then the temperature was increased to 160 °C using 5 °C/min increments. This temperature was maintained for 40 min and then increased using 5 °C/min increments to 180 °C. This temperature was held for 40 min and then the temperature was increased to 200 °C using 5 °C/min increments. The oven was held at 200 °C for 40 min and then the temperature was increased to 210 °C using 5 °C/min increments. This temperature was held for 46 min, with the total run time being 300 min. Chromatographic peaks identified as column bleed peaks did not contribute to the area counts generated by integration of the mass chromatograms.

2.2. Validation of surrogate hydrocarbon mixture (SHM) and total JP-8 calibration curves

The method validation design for SHM and total JP-8 calibration curves was based on the work of Shah et al. [18]. Initial criteria for accepting the validation were accuracy (100 − percent relative error) of better than 80% and a precision (RSD) of less than 20%. For each set of studies a standard calibration curve was constructed for the SHM containing 34 chemicals and another for total JP-8 dissolved in chloroform. Then four concentrations were selected within the range of the calibration curve for the SHM and for total JP-8. These concentrations were prepared in replicates of 5 and then analyzed by GC/MS. This entire procedure was carried out three times. The reliability of the analytical method was determined by calculating the precision and accuracy.

The 34 chemicals selected for validation of the SHM standard curve are listed in Table 1. Individual chemical sources are also listed in Table 1. JP-8 was provided by Dr. David Mattie, Wright-Patterson Air Force Base. Initially 60 hydrocarbons were purchased to assist in the positive identification of individual hydrocarbons found in JP-8. The retention time for each chemical in JP-8 samples was determined by comparison to the corresponding authentic standard. The selection of 34 chemicals from the 60 hydrocarbons was based on their abundance in JP-8, known toxicity information, and ensuring that chemicals representing aromatics, *n*-alkanes, substituted aromatics, naphthenes, and isoalkanes were included in the surrogate hydrocarbon mixture. Each selected compound represented at least 0.15% (w/w) of the mass of neat JP-8. There was little toxicological data available on the individual

Table 1
List of the components used to create the surrogate solution for the method validation

Compound	Purity (%)	Supplier	Retention time (min)	Percent mass in neat JP-8 (%)
Toluene	99.8	Aldrich	42.22	0.15
n-Octane	>99	Sigma	46.50	0.55
n-Ethylbenzene	99.8	Acros	55.21	0.32
m-Xylene	>99	Aldrich	56.51	0.26
p-Xylene	>99	Aldrich	56.70	
o-Xylene	99.5	Fluka	60.84	0.44
n-Nonane	99	Sigma	62.39	2.05
n-Propylcyclohexane	99	Aldrich	70.63	0.78
3-Ethyltoluene	99	Aldrich	75.12	0.69
2-Methylnonane	99	Avocado[a]	76.95	0.59
Mesitylene	99	Acros	76.97	
Pseudocumene	NK	Sigma	82.41	2.05
n-Decane	>99	Sigma	84.00	5.58
1,2,3-Trimethylbenzene	98	Chem Service	88.51	1.55
n-Butylcyclohexane	>99	Acros	92.77	1.17
Indene	NK	Chem Service	93.06	0.27
4-Methyldecane	98.7	Chem Service	97.73	1.24
2-Methyldecane	99.5	Chem Service	98.57	1.88
3-Methyldecane	98.9	Chem Service	100.32	1.92
n-Undecane	99	Sigma	107.70	8.63
1,2,3,4-Tetrahydronaphthalene	98	Chem Service	125.20	0.67
Naphthalene	NK	Supelco	130.22	0.97
n-Dodecane	99	Sigma	134.64	6.73
2-Methylnaphthalene	97	Aldrich	161.32	1.11
n-Tridecane	99	Sigma	163.61	4.92
1-Methylnaphthalene	97	Acros	166.47	0.77
n-Tetradecane	99	Avocado[a]	189.31	3.88
2,6-Dimethylnaphthalene	99	Acros	190.30	0.50
2,7-Dimethylnaphthalene	99	Acros	190.82	0.28
1,3-Dimethylnaphthalene	96	Acros	194.47	0.62
1,6-Dimethylnaphthalene	95	Ultra Scientific	195.54	0.48
n-Pentadecane	99	Avocado[a]	216.91	2.35
n-Hexadecane	99	Acros	241.90	0.83
n-Heptadecane	99	Alfa Aesar[b]	268.81	0.19

NK: not known.
[a] Avocado Research Chemicals.
[b] Johnson Matthey.

components of JP-8, however, data existed for ethylbenzene, xylene, toluene, naphthalene and methylnaphthalene. These compounds were included because they met our initial selection criteria for abundance, fuel composition representation and also allowed for possible correlations to observed health effects from JP-8 exposure based on their toxicity profiles.

To prepare the SHM standard curve, 30 mg of each of the components listed in Table 1 was placed in a single 40 mL volatile organic analysis vial (VWR Scientific, West Chester, PA, USA). Chloroform (Acros Organics, Morris Plains, NJ, USA) was added to this vial to achieve a final volume of 40 mL. The stock SHM was then diluted with chloroform to create a working stock concentration of 250 μg/mL. Serial dilutions were then conducted with chloroform to construct a calibration curve with concentrations of 250, 100, 50, 10, 5, 2.5, and 1.25 μg/mL. Five replicates at nominal concentrations of 1.25, 4.0, 75, and 200 μg/mL were analyzed as well. The concentration of the replicate, assuming no experimental error, is referred to as the *theoretical concentration* and the standard curve derived concentration is referred to as the *experimental concentration*. In a similar fashion to the SHM, a calibration curve for total JP-8 was constructed using neat JP-8 with concentrations of 1500, 1200, 900, 600, 300, 100, 50, 40, and 25 μg/mL. Five replicates at nominal concentrations of 25, 75, 500, and 1000 μg/mL of neat JP-8 in chloroform were analyzed. For both the SHM and neat JP-8, the values determined from the replicate samples were used to calculate the accuracy and precision of the analytical method.

Weighted ($1/X^2$) standard curves for the individual 34 components in the SHM and total JP-8 were created using JMP-IN 5.1 (SAS Institute, Cary, NC, USA). For determining total JP-8, the area of the chloroform peak, which did not interfere with sample peaks, was subtracted from the total peak area and the adjusted total peak area was plotted as a function of concentration. A linear equation best described the relationship between the area under the curve (AUC) for the chromatographic peak(s) of interest and the concentrations tested. For each of the 34 components, over the 3 days of validation, the correlation coefficients for the line were

0.994 or higher and the relative standard deviation of the slopes was between 7.1% and 9.3% ($n = 3$).

To judge the reliability of the validated method for analysis of aerosolized JP-8, both accuracy (100 − percent relative error) and precision (relative standard deviation) were calculated as follows:

$$A = 100 - \left(\frac{|\mathrm{EC} - \mathrm{TC}|}{\mathrm{TC}}\right) \times 100 \quad (1)$$

where A is the accuracy (unitless), EC the experimental concentration (μg/mL) and TC the theoretical concentration (μg/mL).

$$P = \frac{\mathrm{STDDEV_{AUC}} \times 100}{\mathrm{Avg_{AUC}}} \quad (2)$$

where P is the precision (unitless), AUC (μg/mL min) the area under the curve for the chromatographic peak, STDDEV the standard deviation of AUC and Avg the average of AUC.

Interday ($n = 15$) accuracy and precision values were calculated for all validation samples by combining the three batches of intraday data. Accuracy for total JP-8 samples was further verified using a purchased 10 mg/mL reference standard of JP-8 (Supelco) and comparing three 200 μg/mL solutions of the purchased reference JP-8 with predictions from our standard curve of JP-8.

The stability of JP-8 in chloroform was evaluated over a 9-day period, the length of time required for the GC/MS to process each batch of samples created for the method validation. Nine vials containing 200 μL aliquots of 1574 μg/mL of JP-8 were placed on the autosampler, and one vial was analyzed each day. No loss in sample concentration over the 9 days was detected.

2.3. Analyses of chamber atmosphere using the validated method

An inhalation chamber from another laboratory was sampled for aerosolized JP-8. The chamber was originally described by Hays et al. [17] using a DeVilbiss Model #25 ultra-sonic nebulizer and later updated with a DeVilbiss Model 99 ultra-sonic nebulizer [16]. The latter configuration was used for this study. Jet fuel was aerosolized by placing 15 mL of JP-8 (lot 3509) in the nebulizer (Model 25, Somerset, PA, USA). Respiratory tubing connected the nebulizer to the chamber. Altering the distance of this tubing to the chamber opening and adjusting the frequency of the nebulizer were the two parameters that allowed varying levels of aerosol to enter the chamber. All of the 12 port positions used for animal exposures were sealed with the exception of the port used to sample. For these experiments, the aerosol production was manipulated using only the output control knob (frequency) on the nebulizer. Passive entrainment of the jet fuel aerosol/vapor mix and room air was conducted by vacuum (42 L/min). The vacuum was applied to the exposure chamber on the side opposite the fuel source [17].

Aerosolized JP-8 was generated in the inhalation chamber for two 40 min periods and sampled using a handheld pump with an approximate pump flow rate of 100 mL/min as described by Smith et al. [19]. The aerosol portion of the sample was collected on a single glass fiber filter housed in a stainless steel filter holder followed in series by a two-chamber glass coconut charcoal tube, which was used to collect the vapor portion. The glass fiber filters were extracted by emersion in 5 mL of chloroform, and each chamber of the charcoal filter was extracted using 1 mL of chloroform. In all cases, no signals were detected from the extractions of the second chamber, indicating that no breakthrough of JP-8 occurred from the first chamber. Samples were extracted in chloroform for approximately 1 h at room temperature with no heating or agitation and were then transferred into 2 mL GC vials and sealed using Teflon-lined septa screw caps. Samples were then shipped at 0 °C overnight from The University of Arizona to The University of Georgia. Samples were processed immediately. A 1 μL/mL solution of JP-8 was also shipped and compared against a freshly made solution to determine loss during shipping. The analysis revealed that no measurable loss occurred during shipping.

The extraction efficiency of the charcoal tube was determined by spiking the charcoal with known amounts of neat JP-8, extracting with chloroform and then comparing the chromatograms with those of neat JP-8 standards in chloroform. The charcoal was removed from its glass container and placed in a 2 mL vial and spiked with either 1, 3, or 9 μL of neat JP-8. The vials were sealed, and 1 h later briefly opened to add 1 mL of chloroform. After a 1 h extraction time, 200 μL of sample was removed from the vial and placed in a 300 μL vial insert placed in a 2 mL GC autosampler vial and analyzed. Five replicates at each concentration were analyzed. The same method was used for the glass fiber filters, except the extraction was carried out using a 5 mL volume of chloroform.

To determine the concentration of fuel from the chamber samples, calibration curves were prepared for the SHM and total JP-8. The chamber samples were then analyzed and the concentrations calculated using the calibration curve. The chamber concentrations of aerosolized JP-8 as total JP-8 and as 34 individual components (SHM) in the aerosol droplets and in the vapor portion were calculated as follows:

$$\mathrm{Ch} = \frac{\mathrm{mass}}{\mathrm{FR} \times T \times \mathrm{CF}} \quad (3)$$

where Ch is the chamber concentration (mg/m^3), mass the milligram of fuel on glass filter or charcoal tube, FR the pump flow rate (L/min), T the time in min and CF the conversion factor of 1000 L (1 m^3). The pump flow rate used for sampling was 0.100 L/min. The mass of the fuel on the glass fiber or charcoal was calculated as follows:

$$\mathrm{mass} = \mathrm{Cf} \times \mathrm{Vs} \quad (4)$$

where Cf equals the calculated concentration (mg/L) of aerosol or vapor in the chloroform and Vs the solvent volume (0.005 or 0.001 L of chloroform).

For charcoal, the samples were corrected for extraction efficiency. The charcoal tube samples were further diluted to one-tenth of the original concentration for analysis along with the original concentration vials. Both of the coconut charcoal chambers were analyzed to determine breakthrough. JP-8 was detected only in the main chamber of the charcoal tube.

3. Results

3.1. Method validation for surrogate hydrocarbon mixture and total JP-8

The analytical method for the SHM and total JP-8 was deemed successful for the application to quantify aerosolized JP-8. The average accuracy and precision values for the 34 components of the SHM combined were 86.6% or better and 8.0% or better, respectively. The intra- and interday accuracy and precision values for each of the 34 components at the four concentrations are shown in Tables 2 and 3. Using a 3:1 signal-to-noise ratio, the limit of detection of our method was determined to be 1.0 μg/mL for each component. The limit of quantification was set at 1.25 μg/mL using the level at which the accuracy was higher than 80% and the precision lower than 20% for all of the components of JP-8. This level was 1.25 μg/mL for all 34 components of the SHM.

For total JP-8, interday accuracy and precision values were determined using four different concentrations encompassing the range of the calibration curve. The resulting interday accuracy values ($n = 15$) for samples at 25, 75, 500, and 1000 μg/mL were 85.7, 89.3, 88.0, and 84.5%, respectively. The interday precision values ($n = 15$) for neat JP-8 samples at 25, 75, 500, and 1000 μg/mL were 6.7, 4.4, 5.1, and 7.0%, respectively. The intraday accuracy and precision values ranged from 94.8% to 80.4% and 2.41% to 10.5%, respectively. An additional test for the validity of the total JP-8 method involved a comparison to a purchased reference standard of JP-8 (Supelco, Bellefonte, PA). A calibration curve was generated using the JP-8 obtained from Wright-Patterson Air Force Base and then measuring QC points generated from the JP-8 obtained from the new source. These experiments resulted in an average accuracy of 85.1% ($n = 3$). However, it is important to note that JP-8 is produced to a

Table 2
Intraday precision (%) and accuracy (%) values for 34 components of JP-8 ($n = 5$)

Component	1.25 μg/mL		4.0 μg/mL		75.0 μg/mL		200.0 μg/mL	
	Precision	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision	Accuracy
Toluene	4.00	94.39	8.40	87.46	3.71	95.39	1.29	95.39
n-Octane	3.71	95.69	7.88	87.95	3.59	95.70	1.30	95.70
n-Ethylbenzene	3.32	92.43	10.01	88.27	3.41	94.67	1.33	94.67
m-Xylene/*p*-xylene	3.63	91.99	10.16	89.23	3.29	94.61	1.24	94.61
o-Xylene	3.19	90.10	10.54	88.63	3.32	94.50	1.26	94.50
n-Nonane	3.31	92.20	9.88	90.08	3.48	94.74	1.22	94.74
n-Propylcyclohexane	3.18	91.51	10.32	88.64	3.49	94.77	1.23	94.77
3-Ethyltoluene	3.39	89.84	11.29	88.52	3.11	93.64	1.28	93.64
2-Methylnonane/mesitylene	2.93	89.88	10.91	91.60	2.94	92.98	1.16	92.98
Pseudocumene	3.23	89.29	11.29	88.82	2.86	93.57	1.19	93.57
n-Decane	2.82	88.82	10.96	91.84	2.99	93.12	1.16	93.12
1,2,3-Trimethylbenzene	3.16	88.87	11.30	88.93	2.73	93.73	1.20	93.73
Butylcyclohexane	2.55	87.68	10.94	90.28	3.01	93.86	1.20	93.86
Indene	2.78	88.36	11.39	90.43	2.72	92.72	1.09	92.72
4-Methyldecane	2.79	88.34	11.48	91.58	2.79	92.63	1.11	92.63
2-Methyldecane	2.71	87.94	11.25	92.02	2.69	93.12	1.09	93.12
3-Methyldecane	2.84	87.69	11.55	91.69	2.68	92.81	1.10	92.81
n-Undecane	2.55	86.77	11.52	91.34	2.69	92.68	1.09	92.68
1,2,3,4-Tetrahydronaphthalene	2.94	86.95	11.71	89.44	2.46	94.13	1.10	94.13
Naphthalene	2.87	87.75	11.63	89.02	2.50	93.58	1.02	93.58
n-Dodecane	2.67	87.91	11.82	91.04	2.50	93.23	1.01	93.23
2-Methylnaphthalene	3.02	87.66	12.22	86.89	2.22	93.70	1.05	93.70
n-Tridecane	2.32	85.88	12.05	90.42	2.30	93.96	1.04	93.96
1-Methylnaphthalene	2.93	87.32	12.00	88.40	2.04	94.41	1.05	94.41
n-Tetradecane	2.45	86.09	12.24	89.79	1.99	93.78	0.96	93.78
2,6-Dimethylnaphthalene	2.62	87.60	12.30	87.43	1.73	94.71	1.06	94.71
2,7-Dimethylnaphthalene	2.48	87.53	12.00	89.31	1.64	94.82	1.06	94.82
1,3-Dimethylnaphthalene	2.58	87.48	12.23	88.43	1.62	94.69	1.05	94.69
1,6-Dimethylnaphthalene	2.61	87.31	12.24	89.43	1.53	94.86	1.04	94.86
n-Pentadecane	2.59	86.90	12.16	90.66	1.56	93.57	0.97	93.57
n-Hexadecane	2.45	86.50	12.44	88.98	1.52	93.96	0.98	93.96
n-Heptadecane	2.33	87.16	12.31	88.39	1.39	94.79	0.98	94.79

performance standard and not to a compositional standard. Therefore, the Supelco and the US Air Force samples are not equivalent in terms of the concentration of individual components. It appears that by using the area of all of the peaks associated with JP-8, these individual differences may be averaged out. If this were true, then this method may be applicable to determining total JP-8 concentration without regard to the origin of the batch.

3.2. Analysis of chamber atmosphere

The fuel samples collected from the chamber atmosphere were extracted from charcoal (vapor) or glass wool (aerosol) using chloroform. The average percent recovery of fuel mass from the charcoal tubes and glass fiber filters was 87.6% and 100.0%, respectively ($n = 5$). The charcoal tube masses from the chamber samples were adjusted to account for the recovery.

For the two independent 40 min chamber runs, the calculated concentrations of total JP-8 in aerosol and vapor portions were 2013.7 and 821.1 mg/m^3. The total JP-8 concentrations in the aerosol droplets were 191.7 and 52.6 mg/m^3 and the remaining concentrations were vapor (1822 and 768.5 mg/m^3). The aerosol portion of the aerosolized JP-8 represented only 9.5% and 6.4% of the chamber atmospheres, respectively. Fig. 1 depicts representative chromatographs of the aerosol and vapor portions of the chamber atmosphere, neat JP-8 and the SHM. Interestingly, both the aerosol and vapor contained C_{11}–C_{15} (undecane to pentadecane) *n*-alkanes (Tables 4 and 5). As expected the vapor portion was skewed to the lower molecular weight hydrocarbons and the aerosol to the heavier molecular weight hydrocarbons. Comparison of the neat JP-8 chromatograph with that of the SHM chromatograph illustrates that the SHM is representative of the hydrocarbon composition of neat fuel.

In the vapor samples, 21 of the 34 components in the SHM were quantified (Table 4). *n*-Alkanes, specifically nonane (C_9) and decane (C_{10}), represented the largest fraction of the vapor samples for both runs. The 21 components that were quantified accounted for about 68–70% of the vapor sample mass. In the aerosol samples, 7 of the 34 components in the SHM were quantified (Table 5). These seven *n*-alkanes represented about 40–44% of the aerosol mass. Tetradecane (C_{14}) and pentadecane (C_{15}) accounted for a majority of the identified *n*-alkanes in the aerosol. The composition or 'fingerprint' of the identifiable components in JP-8 vapor and aerosol for

Table 3
Interday precision (%) and accuracy (%) for 34 components of JP-8 ($n = 15$)

Component	1.25 μg/mL		4.0 μg/mL		75.0 μg/mL		200.0 μg/mL	
	Precision	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision	Accuracy
Toluene	5.67	89.40	6.66	86.57	7.55	93.22	4.94	89.49
n-Octane	5.48	86.55	6.46	86.57	7.16	93.53	4.95	89.54
n-Ethylbenzene	5.51	90.84	7.13	88.52	6.92	93.12	4.23	89.45
m-Xylene/*p*-xylene	5.61	90.69	7.15	89.70	6.60	93.18	4.01	90.48
o-Xylene	5.48	90.85	7.34	88.99	6.44	93.21	4.04	89.21
n-Nonane	5.70	88.49	7.22	89.76	6.55	93.14	4.29	89.40
n-Propylcyclohexane	5.54	90.04	7.32	88.60	6.52	93.34	4.24	88.62
3-Ethyltoluene	5.87	91.73	7.48	89.65	6.02	93.04	3.55	88.40
2-Methylnonane/mesitylene	5.47	90.22	7.45	92.71	5.80	92.68	3.30	91.06
Pseudocumene	5.66	91.81	7.49	89.76	5.60	93.58	3.03	89.67
n-Decane	5.48	90.24	7.53	92.58	5.77	92.57	3.19	89.20
1,2,3-Trimethylbenzene	5.58	91.89	7.53	90.38	5.54	93.11	2.68	88.81
Butylcyclohexane	5.25	91.64	7.48	90.96	5.65	93.11	2.79	86.36
Indene	5.63	91.35	7.50	91.95	5.40	92.79	2.53	90.57
4-Methyldecane	5.84	91.04	7.72	92.74	5.31	92.84	2.53	88.57
2-Methyldecane	5.73	90.70	7.65	93.03	5.08	92.80	2.39	88.74
3-Methyldecane	5.84	90.95	7.65	92.85	5.05	92.61	2.31	88.51
n-Undecane	5.84	90.82	7.76	92.53	4.95	92.43	2.26	87.70
1,2,3,4-Tetrahydronaphthalene	5.74	91.82	7.53	91.41	4.57	93.85	1.85	88.64
Naphthalene	5.73	91.85	7.61	90.90	4.71	93.52	1.76	89.01
n-Dodecane	6.30	91.75	7.72	92.77	4.64	92.77	1.88	88.43
2-Methylnaphthalene	6.30	92.08	7.78	89.21	4.32	93.73	1.57	88.65
n-Tridecane	6.27	91.39	7.89	92.12	4.32	93.19	1.67	87.50
1-Methylnaphthalene	6.09	91.88	7.71	90.59	3.95	94.02	1.36	89.35
n-Tetradecane	6.15	91.71	8.01	91.79	3.85	93.02	1.39	88.29
2,6-Dimethylnaphthalene	6.05	92.15	7.82	89.76	3.50	94.30	1.21	90.10
2,7-Dimethylnaphthalene	5.88	92.01	7.62	91.48	3.29	94.29	1.17	90.45
1,3-Dimethylnaphthalene	5.99	92.13	7.80	90.65	3.09	94.24	1.19	90.07
1,6-Dimethylnaphthalene	5.93	92.05	7.76	91.63	2.91	94.42	1.15	90.31
n-Pentadecane	6.17	89.23	7.96	92.43	2.77	92.81	1.22	89.16
n-Hexadecane	5.88	92.02	8.00	91.23	2.75	93.03	1.21	89.20
n-Heptadecane	5.86	92.22	7.91	90.73	2.72	93.76	1.24	89.42

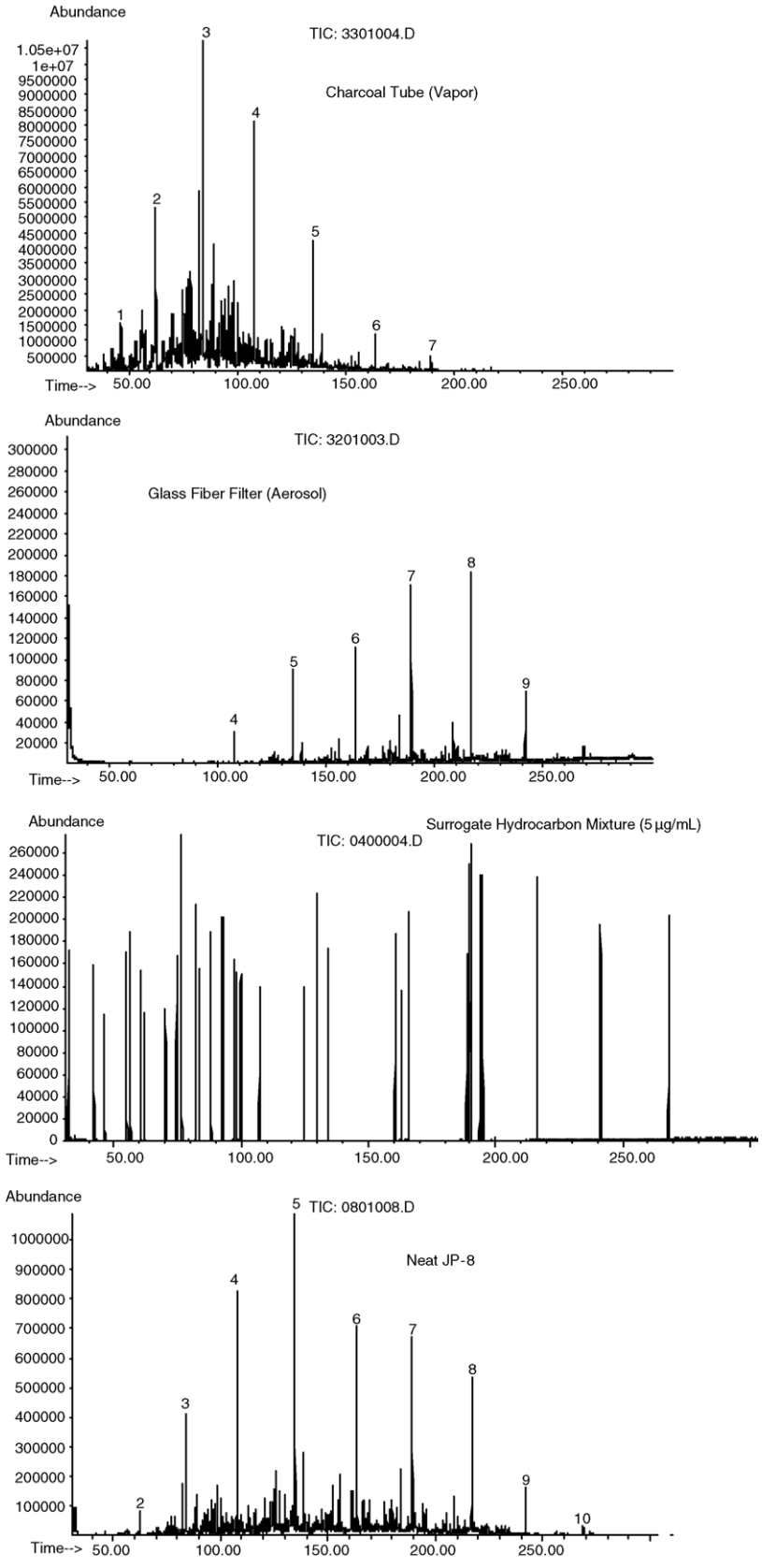

Fig. 1. Chromatographic output for visual comparison of the vapor portion, aerosol portion, surrogate hydrocarbon mixture, and neat JP-8: (1) *n*-octane, (2) *n*-nonane, (3) *n*-decane, (4) *n*-undecane, (5) *n*-dodecane, (6) *n*-tridecane, (7) *n*-tetradecane, (8) *n*-pentadecane, (9) *n*-hexadecane and (10) *n*-heptadecane.

Table 4
Calculated total vapor concentrations and individual component vapor concentrations of aerosolized JP-8 in a chamber

Component	RUN 1 JP-8 vapor concentration 1822.00 mg/m^3		RUN 2 JP-8 vapor concentration 768.50 mg/m^3	
	Component concentration (mg/m^3)	Percent mass of sample	Component concentration (mg/m^3)	Percent mass of sample
Toluene	17.61	0.97	7.00	0.91
n-Octane	148.67	8.16	60.26	7.84
n-Ethylbenzene	58.08	3.19	22.32	2.90
m-Xylene/*p*-xylene	30.47	1.67	10.97	1.43
o-Xylene	41.57	2.28	15.25	1.98
n-Nonane	245.56	13.48	92.39	12.02
n-Propylcyclohexane	83.15	4.56	31.22	4.06
3-Ethyltoluene	44.53	2.44	18.08	2.35
2-Methylnonane/mesitylene	33.56	1.84	13.22	1.72
Pseudocumene	35.47	1.95	14.39	1.87
n-Decane	214.31	11.76	88.59	11.53
1,2,3-Trimethylbenzene	45.72	2.51	18.78	2.44
Butylcyclohexane	44.15	2.42	18.23	2.37
4-Methyldecane	33.67	1.85	14.07	1.83
2-Methyldecane	35.58	1.95	14.66	1.91
3-Methyldecane	30.73	1.69	12.99	1.69
n-Undecane	116.94	6.42	48.56	6.32
n-Dodecane	35.99	1.98	15.27	1.99
n-Tridecane	12.89	0.71	7.60	0.99
n-Tetradecane	5.9	0.32	3.95	0.51
n-Pentadecane	1.78	0.10	1.51	0.20
Percent of total mass		71.3		68.0

Samples were collected on charcoal tubes.

Table 5
Calculated total aerosol concentrations and individual component aerosol concentrations of aerosolized JP-8 in a chamber

Component	RUN 1 JP-8 Aerosol Concentration 191.70 mg/m^3		RUN 2 JP-8 Aerosol Concentration 52.59 mg/m^3	
	Component concentration (mg/m^3)	Percent mass of sample	Component concentration (mg/m^3)	Percent mass of sample
n-Undecane	7.79	4.07	Below LOQ	–
n-Dodecane	8.83	4.61	1.99	3.79
n-Tridecane	13.15	6.86	3.47	6.61
n-Tetradecane	21.89	11.42	6.18	11.76
n-Pentadecane	20.58	10.74	6.31	12.00
n-Hexadecane	9.25	4.82	3.27	6.21
n-Heptadecane	2.41	1.26	Below LOQ	–
Percent of total mass		43.8		40.4

Samples were collected on glass fiber filters.

RUN 1 (2013.7 mg/m^3) and RUN 2 (768.5 mg/m^3) were similar except that *n*-undecane and *n*-heptadecane in aerosol were below the LOQ in RUN 2. The individual component concentrations in RUN 2 were lower than in RUN 1 as would be expected based on the difference in total concentrations.

4. Discussion

In this study, we developed the first analytical method for simultaneous separation and identification of total JP-8 and up to 34 individual components in JP-8. The accuracy and precision calculations for the hydrocarbon components of JP-8 and the total JP-8 were of sufficient quality to allow for interrogation of the composition of the vapor and aerosols from an animal exposure chamber. Using this analytical method for analyses of the chamber samples of aerosolized JP-8, about 40% of the hydrocarbon mass of the aerosol portion was identified and quantified and about 70% of the vapor portion. However, there are still significant peaks in the vapor and especially the aerosol that are not accounted for in the 60 standards evaluated in the study. From the EI mass spectra these compounds appear to be higher molecular weight branched hydrocarbons. Unfortunately, these compounds were not available as synthetic standards making their identification very difficult due to the substantial number of positional isomers.

Since this is the first validated method for the analysis of JP-8, there are no other methods available for comparison of accuracy or precision. However, an Air Force (AF)

technical report (TR) did describe hydrocarbon composition of aerosolized JP-8 in a chamber at ExxonMobil Biomedical Sciences Inc. [20]. The aerosol generation system was different than the system used in this study, however, the chamber sampling methodology was similar to the present study. The samples for this study were analyzed using gas chromatography with a flame ionization detector but there are no details describing the accuracy, precision, recovery or linearity of the method. The AF TR provided information on five chamber runs with total JP-8 concentrations ranging from about 600 mg/m^3 up to about 3600 mg/m^3, compared to the present study of about 800 and 2000 mg/m^3. The measured aerosol fraction of the aerosolized JP-8 increased with chamber concentration, ranging from non-detectable at the lower concentration to 35% in the higher concentration. For comparable chamber concentrations, the aerosol fractions in the present study were less (6–10%) than reported in the AF TR (15–25%), probably due to the differences in the aerosol generation systems. In the AF study, the most abundant hydrocarbons identified in the aerosol fraction were *n*-alkanes ranging from *n*-decane (C_{10}) to *n*-hexadecane (C_{16}) compared to the present study, which showed measurable levels of *n*-alkanes ranging from *n*-undecane (C_{11}) to *n*-hexadecane (C_{16}). The mean aerodynamic droplet size of the aerosol was 0.25 μm for the AF study and for the chamber used in the present study, 1.7 μm [16]. In the vapor portion, the three most abundant hydrocarbons measured in the AF study were *n*-decane, *n*-undecane, and *n*-dodecane in four of five runs and *n*-octane, *n*-heptane, and isooctane for one run. In the present study, the three most abundant hydrocarbons in the vapor were *n*-nonane, *n*-decane, and *n*-undecane. It is worth noting that in both the AF and the present study only alkanes were observed in the aerosol portion of the exposure chambers. So even though no limit of quantitation are provided in the AF TR their method does not appear to be more sensitive than then current method. An important result of these two studies is that while the alkanes may not be the species that cause the toxic effects observed in animal exposures, as the major components present, they clearly merit further investigation.

In a published study of AF personnel exposed to jet fuel, Pleil et al. [21] reported that the breath of fuel exposed AF personnel contained several hydrocarbons found in JP-8 (*n*-octane, *n*-nonane, *n*-decane, *n*-undecane and *n*-dodecane, benzene, toluene, ethylbenzene, and xylenes). These particular hydrocarbons were also measured in the ambient air where the workers performed their duties. In the current study we were able to quantitate all of these components and eleven additional components in the vapor portion of the aerosolized JP-8. The only exception was benzene. We did observe a very small quantity of benzene in the neat JP-8 (data not shown) that we used to generate our aerosolized samples. Since we were able to detect all of the other species observed by Pleil et al. plus many additional compounds, we speculate that the lot of JP-8 used in the our study contained a lower initial amount of benzene.

This study is part of a research project to develop a physiologically based pharmacokinetic (PBPK) model for JP-8 in the rat. PBPK models describe the uptake, distribution, metabolism and elimination of chemicals in the body [22]. Usually these models are developed for single chemicals or a few chemicals [23]. In this case we are attempting to develop a PBPK model for a large suite of hydrocarbons that constitute JP-8. The approach for PBPK model development is to select specific hydrocarbons that represent different classes of hydrocarbons and track these individual hydrocarbons in the body as 'marker chemicals' for JP-8 exposure [24]. The data in the present study and that of the AF TR provide important information for selecting 'chemical markers' and documenting that aerosol deposition in the lung is an important dosimetry factor for high concentrations of aerosolized JP-8.

In summary, the first validated analytical method has been developed to quantify total JP-8 and several individual abundant hydrocarbons found in aerosolized JP-8 vapor and aerosol droplets. This method can be used to quantify inhalation chamber exposures for animals. In addition, this method can be applied to the development of future analytical methods for the determination of JP-8 hydrocarbons from tissues and blood of animals exposed to aerosolized JP-8.

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