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Optimized determination of trace jet fuel volatile organic compounds in human blood using in-field liquid–liquid extraction with subsequent laboratory gas chromatographic–mass spectrometric analysis and on-column large-volume injection

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Abstract

A practical and sensitive method to assess volatile organic compounds (VOCs) from JP-8 jet fuel in human whole blood was developed by modifying previously established liquid–liquid extraction procedures, optimizing extraction times, solvent volume, specific sample processing techniques, and a new on-column large-volume injection method for GC–MS analysis. With the optimized methods, the extraction efficiency was improved by 4.3 to 20.1 times and the detection sensitivity increased up to 660 times over the standard method. Typical detection limits in the parts-per-trillion (ppt) level range were achieved for all monitored JP-8 constituents; this is sufficient for assessing human fuels exposures at trace environmental levels as well as occupational exposure levels. The sample extractions are performed in the field and only solvent extracts need to be shipped to the laboratory. The method is implemented with standard biological laboratory equipment and a modest bench-top GC–MS system. © 2001 Published by Elsevier Science B.V.

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1. Introduction

Human exposure assessment to various environmental and occupational contaminants is generally performed through detailed study of ambient concentrations in air, water, food, and any other potential sources. These data are then used in conjunction with parameters such as breathing rate, ingestion volumes, and dermal contact areas to estimate internal dose. More direct methods employ the assay of bodily fluids or tissues such as blood, urine, and breath to confirm exposure and measure contaminant dose [1–4]. Ambient exposures and their biological expression are then used for establishing models to predict health outcomes and risks.

Our laboratory has been involved in collaborative study with the US Air Force in method development for human exposure assessment to JP-8, a jet fuel used in military and civilian aviation [5]. Several recent studies have indicated a toxic effect of JP-8 on the immune system [6], postural balance [7], pulmonary function [8], embryo growth [9], kidney function [10] and skin irritation [11]. Like other

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petroleum distillate fuel, JP-8 is a complex mixture of aliphatic and aromatic volatile and semi-volatile organic compounds (VOCs or sVOCs). Based on its relatively high volatility (as compared to diesel fuels, pesticides, and polyaromatic hydrocarbons, for instance), the exposure assessment of JP-8 has been performed through the measurement of breath levels using the technology developed by Pleils and coworkers [12–17]. JP-8 exposure of all subjects was determined by collecting and analyzing the breath samples from various groups of air force personnel, which showed breath levels of JP-8 ranging from slight elevations compared to the control cohort to more than 100 times the control values [18]. For these non-invasive breath measurements to become more useful for toxicological interpretation, we require a direct (rather than calculated) link to actual blood levels. Such in vivo data for alkanes and some aromatic constituents of JP-8 are not currently available; as such we have been developing simple and sensitive methodology for assaying JP-8 in the human blood matrix.

Measurement of a variety of VOCs in blood has been reported mainly using the technologies of purge-and-trap gas chromatography (GC) and solid-phase microextraction (SPME) [19–27]. We chose another alternative, liquid–liquid extraction–GC, because it allows in-field stabilization of the analytes into a compatible solvent prior to shipping and storage, avoids having to routinely bring potentially infectious biohazard materials (human whole blood) into the laboratory, and is well suited for both volatile and semi-volatile compounds. We originally started this research using liquid–liquid extraction of a series of representative components of JP-8 including C_9–C_{15} *n*-alkanes, benzene and toluene from a physiological phosphate-buffered saline (PBS) solution, a surrogate for blood. We generated various concentrations in expected ranges of occupational exposures and analyzed the condensed extracts with conventional GC–mass spectrometry (MS). This initial work is the subject of a previous article in this journal [28].

The previous results have demonstrated the practicality and applicability of liquid–liquid extraction; however, when the method was applied directly to animal blood products and human blood, lower recovery efficiencies were experienced. Additionally, we expect the actual human blood concentrations of JP-8 and other analytes to range well below the established sensitivity of the previous technique especially for incidental or environmental exposure. Therefore, optimization of both extraction and analysis techniques to improve sensitivity is required for the assessment of human exposure to JP-8 in blood.

This paper presents a practical and sensitive field method for collecting blood and creating sample extracts, as well as a specific laboratory process and analysis for measuring exogenous compounds. The final method is sensitive enough to assess typical environmental levels as well as the greater occupational exposure levels. We have applied this technology to extract and analyze VOCs with a wide variety of physical and chemical properties in human blood samples spiked with various levels of JP-8 jet fuel. This work demonstrates the general applicability of this methodology for exposure assessment of various organic pollutants in blood.

2. Experimental

2.1. Materials

Pentane and acetone solvents were purchased from Burdick & Jackson (Muskegon, MI, USA) as GC–MS grade. The reagents benzene, toluene, ethylbenzene, *o*-, *m*-, *p*-dimethylbenzene, 1,3,5-trimethylbenzene, naphthalene, nonane, decane, undecane, dodecane, tridecane, tetradecane and pentadecane were purchased from PolyScience (Niles, IL, USA) as analytical standards. Deuterated dodecane (dodecane-d_{26}), deuterated benzene (benzene-d_6) and deuterated pentadecane (pentadecane-d_{32}) were purchased from Cambridge Isotope Labs (Andover, MA, USA). Reagent PBS tablets were purchased from Sigma (St. Louis, MO, USA).

The PBS solution was prepared in this laboratory by dissolving one PBS tablet in 200 ml of deionized water (0.01 *M* phosphate, 0.0027 *M* potassium chloride, and 0.137 *M* sodium chloride, pH 7.4) and NaCl-saturating by adding NaCl until no more can be dissolved; sulfuric acid and sodium chloride were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Jet fuel samples were obtained directly from various aircraft fuel tanks at more than 10 air force

bases, stored in refrigerated glass vials, and used as analytical development samples. For use with blood and surrogate spikes, the primary jet fuel sample was collected directly from the fuels facility at Robins Air Force Base (Warner-Robins, GA, USA) with a 1-l evacuated SilcoCan (Restek, Bellefonte, PA, USA) that was submerged in JP-8, opened, allowed to fill completely, and sealed while still below the fuel surface. This primary JP-8 standard was kept stable in the sealed cannister with essentially zero vapor headspace. Bovine plasma was obtained from Pel Freez (Rogers, AR, USA). Human whole blood (treated with heparin) was purchased from American Red Cross (Charlotte, NC, USA) and stored in a −20°C freezer.

2.2. Instrumentation

2.2.1. GC–MS apparatus and conditions

Chromatographic analysis was achieved with an HP 5890 Series II gas chromatograph (Hewlett-Packard, Santa Clarita, CA, USA) directly connected to an HP 5971A mass-selective detector. The system was controlled by an MS Chemstation (Windows 3.1; Hewlett-Packard). A deactivated pre-column (10 m×0.32 mm I.D., Alltech Associates, Deerfield, IL, USA, or 10 m×0.53 mm I.D., Hewlett-Packard) was used to accommodate the direct cold on-column injection of sample; the separation was made with a fused-silica capillary column, 30 m×0.25 mm I.D., coated with 100% dimethyl polysiloxane (1.0 μm thickness, Rtx-1, Restek). The oven temperature program for analysis was 35°C (with small-volume injection)×5 min or 30°C (with large-volume injection)×8–11 min and then 3°C/min to 200°C (VOCs) or 300°C (JP-8). About 1.5 μl of sample was directly injected on the column for the standard method, or up to 50 μl for large-volume on-column injection. Helium was used as the carrier gas (inlet pressure, 100 kPa when the deactivated column was 10 m×0.32 mm I.D. and 55 kPa when the deactivated column was 10 m×0.53 mm I.D.). The MS conditions were as follows: the source temperature was 250°C; the instrument was operated in full-scan mode (44–350 u) with electron impact ionization; parameter values were optimized for maximum sensitivity. For some sensitivity comparison experiments such as selective ion monitoring (SIM)/full scan, samples were analyzed using the SIM mode through which we acquired molecular ions for the selected compounds of interest.

2.2.2. Centrifugation

A centrifuge (Marathon 21K/BR; Fisher Scientific, Pittsburgh, PA, USA) was used to separate pentane solution from biological media (plasma or blood) with an eight-place fixed-angle rotor. Samples were separated at 1600 *g* in 28-ml centrifuge vials.

2.3. Standard curves

Standard curves were made for standard compounds benzene, toluene, nonane, decane, undecane, dodecane, tridecane, tetradecane, and pentadecane. Dodecane-d_{26} was used as an internal standard. Solutions of the standard compounds were prepared by dissolving the compounds in acetone as 100 ng/μl and diluted to 10, 7, 4, 1, 0.7, 0.4 and 0.1 ng/μl with pentane. Internal analytical standard dodecane-d_{26} (2000 ng/μl, 10 μl) was added to each standard solution (200 μl) before injecting it into the GC system. This internal standard solution was used purely as an instrumental quantitation standard to assure that any changes in performance were not misconstrued as a difference in recovery. Peak area ratios for each compound monitored to the internal analytical standard were obtained from GC–MS analysis of each compound at different concentrations (ng/μl) and were used to construct the standard curves. Linear regression from Prizm (GraphPad Software, San Diego, CA, USA) was used to analyze the relationship between the two variables with 95% confidence intervals.

2.4. Preparation and extraction of JP-8 spiked bovine plasma

The initial spike solutions were prepared by dissolving JP-8 in acetone to a series of specific concentrations; blank solvent was used as a control. Then 200 μl of each of the above prepared solutions was mixed with 3 ml of bovine plasma followed by the addition of sodium-chloride-saturated PBS solution (3 ml) to achieve synthetic sample concentrations of 0.16%, 0.016% and 0.0032% JP-8 in 6 ml volume. Some tests were performed with addition of

two drops of sulfuric acid to precipitate proteins. After adding 6 ml pentane to each sample, the resulting solutions were extracted by hand-shaking for 1 min. After extraction, the mixture was then centrifuged at 1600 *g* for 30 min. The organic phase (top) was then separated from the aqueous phase and dried over anhydrous sodium sulfate. After separation from the drying agent, the remaining solutions were evaporated in a Kuderna–Danish (K–D) evaporator at 40–45°C. The evaporation process was stopped when 200 μl of solution was left.

2.5. Optimizing the extraction technique

Experiments for optimizing the volume of extracting solvent and the time of extraction were conducted by extracting a mixture of representative VOCs in JP-8, including benzene, toluene, nonane, decane, undecane, dodecane, tridecane, tetradecane and pentadecane in plasma with pentane. By following the general procedure in Section 2.4, different volumes of solvent (6, 12 and 18 ml) were tested for optimal recovery; the tests also compared repeated 6 ml extraction of the same samples. Effect of extraction time was also tested; the hand-shaking technique was applied for 1, 5 and 20 min. The recovery efficiencies for each compound analyzed were compared among the volume and time experiments.

2.6. Large-volume injection

To accommodate larger sample volumes, the retention gap (pre-column) was changed to 10 m× 0.32 or 0.53 mm I.D. as mentioned in Section 2.2. The solutions prepared for this study were representative VOCs in pentane (1.0 ng/μl) and JP-8 in pentane (0.1%, v/v). VOC solutions of 2, 10, 25 and 50 μl of the same concentration and JP-8 solution of 10 and 40 μl of the same concentration were injected. The peak areas were analyzed on the GC–MS system by using both full-scan and SIM (molecular ion) modes.

2.7. Extraction of JP-8 from human whole blood

Human whole blood was stored at −20°C and thawed before the experiment. The JP-8 solutions were prepared by dissolving JP-8 in acetone and diluting with acetone to concentrations of 0.5, 0.1, 0.05, 0.01 and 0.005%; then 200 μl of each solution was spiked into a blood–NaCl-saturated PBS (50:50) solution to achieve JP-8 concentrations of 0.016, 0.0032, 0.0016, 0.00032 and 0.00016%, respectively. These solutions were extracted by following the general procedure in Section 2.4; blank solvent was used as the control. All biological materials and extracts were treated as potentially infectious by following the OSHA standards for bloodborne pathogens.

2.8. Calculation of recovery efficiency

The control solutions of VOCs or JP-8 were prepared in pentane at concentrations corresponding to those of the sample solutions. The solutions were immediately stored in a refrigerator after their preparation. Just before GC–MS analysis, each sample was mixed with 10 μl of standard dodecane-d_{26} solution of 2000 ng/μl. An aliquot of these samples was injected directly onto the GC column. Analysis using both the SIM and the full-scan modes was performed for each sample. The relative area to internal analytical standard for each compound in sample solution was compared with the relative area to internal analytical standard for each compound in the control solution of the same concentration. The recovery was thus calculated as follows:

$$\text{Recovery (\%)} = \frac{(\text{VOC}_S/\text{I.S.}_S)}{(\text{VOC}_C/\text{I.S.}_C)} \times 100 \qquad (1)$$

where VOC_S = peak area for sample VOC, I.S._S = peak area for sample internal analytical standard, VOC_C = peak area for control VOC and I.S._C = peak area for control internal analytical standard.

3. Results and discussion

In previous studies of liquid–liquid extraction of VOCs from blood surrogate PBS solution, both pentane and dichloromethane (DCM) were used as extracting solvents and showed slightly different effects on the recovery efficiency of aromatic compounds [28]. For this new work with human blood, we chose pentane primarily because the DCM layer

resides on the bottom of the vial after centrifugation which complicates its separation from the blood precipitate.

We confirmed that a series of VOCs including benzene, toluene, nonane, decane, undecane, dodecane, tridecane, tetradecane, and pentadecane represent JP-8 well and were selected for monitoring and quantitative analysis on GC–MS. We also found, as discussed later, that we could recover other minor constituents of JP-8 such as ethylbenzene, *m*,*p*-xylene, *o*-xylene, 1,3,5-trimethylbenzene and naphthalene.

3.1. Linearity and curve fitting

The calibration curve was made for the representative VOCs of JP-8 monitored by GC–MS analysis. The sample solutions were made in pentane over the concentration range 0.1–10 ng/μl. The calibration curves were shown to be linear. These results confirmed that linear regression equations could be generated from each standard curve for each compound.

3.2. Liquid–liquid extraction of JP-8 from bovine plasma

Liquid–liquid extraction of JP-8 spiked in blood product bovine plasma was first conducted following the procedure previously developed using pentane as extracting solvent [28].

We used bovine plasma rather than human whole blood for the initial optimization experiments because it is more readily available and presents a lower biohazard risk. GC–MS analysis demonstrated that all characteristic compounds monitored were extractable from bovine plasma and that the chromatograms were qualitatively identical to the samples from PBS solution. The quantitative analysis of the GC–MS chromatogram (according to Eq. (1)) showed that the recovery efficiency of the process had decreased up to 20 fold at plasma concentrations of 0.016% (v/v) JP-8 compared to that from PBS solution especially for the alkanes with longer chains. Standard GC–MS analysis techniques (with detection limits of 0.06 to 0.6 ng on-column depending on compound) were found to require a minimum concentration in the range of 3.8 to 1110 ng/μl (ppm) VOCs in plasma to reach the detection limit. This is not sufficient for non-exposed control or low-exposure subject assays. These results indicate that improvement of both extraction recovery efficiency and detection sensitivity are necessary to lower the detection limit of extractable concentration of VOCs in JP-8 in blood.

3.3. Improvement of recovery efficiencies from blood product

Biological fluids like plasma and whole blood are more complex media than a clear buffer solution like PBS and contain tissues and macromolecules such as lipids and proteins to which the extracted compounds tend to bind [29]. To break down the possible interaction between the macromolecules and VOCs in JP-8, we tested a method wherein two drops of concentrated sulfuric acid (H_2SO_4) were added to the plasma solution spiked with JP-8 before extraction.

Immediately after the addition of H_2SO_4, massive precipitation was formed. When extracted with pentane, a thick emulsion was produced and the plasma–pentane mixture became an immobilized gel that could not be broken down effectively even after centrifugation. This caused the extraction process to become very difficult and much less efficient. In addition, co-precipitation of the analytes with blood proteins could also be among the factors which caused the losses during the extraction process.

The experiments performed without adding sulfuric acid eliminated the formation of protein precipitation, however, emulsion formation was still observed during the extraction of plasma solution with pentane by high-energy vortexing. Thus, avoiding emulsion formation during extraction became critical for using the liquid–liquid extraction method to extract JP-8 from plasma and blood.

It has been reported that saturating the aqueous phase with various salts, for instance, sodium chloride, will encourage partitioning of ionic compounds into organic phases [30]. The other desirable effect of doing this is reduced occurrence of emulsions [30]. In addition, a high concentration of electrolytes helps to change the protein conformation and reduce the affinity to bind analytes. In our study, it was found that first diluting the blood or plasma with

sodium-chloride-saturated PBS in a 1:1 ratio before extraction reduced the incidence and severity of emulsion formation. Subsequent centrifugation served to completely separate the pentane from the plasma or blood. The recovery efficiency of liquid–liquid extraction listed in Table 1 showed about a 2–3-fold improvement with the modified procedure. Similar to previous observations, the recovery efficiency of benzene was found to be somewhat variable. Because benzene has a relatively high vapor pressure and lower boiling point than the other constituents of JP-8, we ascribe this variability in quantitation to subtle changes in the manual injection techniques and temperatures in the GC–MS analysis as well as to the subtle variability of room temperature changes during the evaporation/concentration process.

Experiments described in Section 2.5 resulted in an overall optimized pentane extraction volume that doubles the total sample volume, i.e., a 1:1 dilution. For example, a typical 7 ml blood sample would be initially mixed with 7 ml sodium chloride saturated PBS and then extracted with 14 ml of pentane. We also found that repeat extraction did not improve efficiency.

For time of extractive handshaking, we found that 20 min was an appropriate overall optimum value. These generalized results are an excellent "best compromise" for the wide volatility range of analytes in JP-8.

More specifically, we found in previous work that the major decreases in recovery efficiency from PBS solution occurred during the evaporation step in direct relation to analyte volatility [28]. When this was tested using bovine plasma, we confirmed that there was no significant difference in the recovery efficiencies for the less volatile compounds such as pentadecane, tetradecane, and tridecane and that the loss from using less extraction solvent was equally compensated by the gain from evaporation of less solvent volume. When the samples were extracted once with a 3:1 ratio of solvent, we observed a slight decrease in recovery efficiency indicating that the losses from the evaporation step start to dominate the gains from the extraction step. For the more volatile compounds, the recovery efficiencies from the extraction with a 1:1 ratio of pentane are slightly higher than those from greater ratios. Apparently, evaporation factors played a more important role than extraction factors on the recovery of more volatile compounds during the liquid–liquid extraction process. Similarly, extraction handshaking time optimization was subject to compound volatility. We tested 1, 5 and 20 min times and found that significant improvement of recovery efficiency was obtained by increasing the extraction for the less volatile compounds; however, the longest extraction time (20 min) slightly reduced the recovery efficiency for the more volatile compounds. These results showed that extraction of VOCs from plasma with a 1:1 ratio of solvent was the optimal volume and 20 min was the optimal value for extraction handshaking for efficient extraction of VOCs from JP-8 with a wide range of physical and chemical properties. If benzene quantitation is of paramount importance, then the 1 min shaking time would be the best choice.

Table 1
Recovery efficiency of liquid–liquid extraction of JP-8 (0.016%) from bovine plasma before and after the technical modification to reduce emulsion formation

Compound	Recovery efficiency±S.E.[a] (%)	
	Before	After
Pentadecane	2.0±0.5	4.5±1.2
Tetradecane	1.2±0.2	4.5±1.2
Tridecane	1.6±0.2	5.2±1.8
Dodecane-d_{26}	100	100
Dodecane	2.3±0.2	6.5±2.2
Undecane	4.9±0.4	13.4±3.4
Decane	9.3±0.8	20.7±5.3
Nonane	22.0±2.0	23.9±6.1
Toluene	11.5±2.3	41.1±22.9
Benzene	35.5±7.4	24.2±16.4

[a] S.E. = Standard error.

Overall, optimization of both extraction time and the volume of solvent for extraction improved the recovery efficiency by 3–5 times, depending on the compounds analyzed. Coupled to the improvement obtained from dilution with NaCl-saturated PBS, we found a 4.3 to 20.1 times increase in recovery efficiency as shown in Table 2. Again, somewhat variable results were obtained for benzene.

3.4. Improvement of the detection sensitivity with large-volume injection

In trace analysis, the introduction of more sample volume is a simple and efficient way to increase

Table 2
Recovery efficiency of liquid–liquid extraction of VOCs from bovine plasma with modified extraction time (20 min) and amount of extracting solvent (6 ml×1 time)

Compound	Recovery efficiency±S.E.[a] (%)		
	6 ml×3×1 min	6 ml×1×1 min	6 ml×1×20 min
Pentadecane	4.1±0.6	3.9±0.3	13.0±0.3
Tetradecane	2.3±0.3	2.1±0.3	12.1±0.5
Tridecane	2.5±0.4	2.4±0.1	9.2±0.2
Dodecane-d_{26} (I.S.)	100	100	100
Dodecane	2.7±0.2	2.9±0.4	12.1±0.3
Undecane	5.2±0.3	5.4±0.7	26.4±4.2
Decane	8.2±0.3	9.0±1.1	30.0±1.1
Nonane	10.6±0.3	13.8±1.3	40.8±1.1
Toluene	8.6±0.3	14.3±0.2	36.7±1.5
Benzene	4.6±0.2	8.9±0.3	1.2±0.2

[a] S.E.=Standard error.

detection sensitivity as long as the separation step and detectors are not overwhelmed by excess matrix material. In GC several techniques are available to perform large-volume injections (LVIs) [31–34]. These include on-column injection with the use of retention gap, loop-type interface, and ‘‘programmed temperature vaporizer’’ (PTV). As described in Section 2.6, we developed a method for accommodating excess solvent using a 10 m×0.53 mm I.D. retention gap where the solvent is subsequently vaporized and stripped from the analytes of interest. This technique was first applied to inject samples of VOCs (1 ng/μl) in pentane at 2, 10, 25 and 50 μl. We determined that setting the injection temperature and initial oven temperature at 30°C and using a solvent delay of 4–11 min (depending on the volume injected) allowed the complete vaporization of solvent before eluting the monitored VOCs. We could not quantitatively recover benzene with injections greater than 10 μl.

Analyte peak areas from different injection volumes are listed in Table 3. From this table, we can see that, in general, the peak area of each compound monitored is proportional to the volume injected (with the exception of benzene). The loss of benzene with the largest volume injections was believed to be due to its similar volatility to pentane; presumably benzene was swamped by the solvent front and thus partially eluted out of the GC–MS system before the detector could be turned on. The general results demonstrate that the large-volume injection technique improved the detection limit by a factor of about 33 compared to the 1.5-μl injection used in standard techniques. Fig. 1 shows representative GC–MS chromatograms of JP-8 in pentane injected at 10 μl and 40 μl. We observed no significant loss of separation efficiency and could identify all the compounds monitored except benzene, as mentioned above.

3.5. Liquid–liquid extraction of JP-8 from human whole blood

The optimized procedure was applied to human whole blood; the concentrations of JP-8 in blood ranged from 0.032 to 0.00064% (v/v) and were always diluted with 1:1 sodium-chloride-saturated PBS solution to achieve 0.016 to 0.00032% (v/v).

Table 3
Peak areas of analytes from large-volume on-column injection of VOC solutions in pentane

	VOC solution (1.0 ng/μl)			
	2 μl	10 μl	25 μl	50 μl
Pentadecane	$1.85\cdot10^7$	$6.9\cdot10^7$	$25.4\cdot10^7$	$60.0\cdot10^7$
Tetradecane	$1.65\cdot10^7$	$6.4\cdot10^7$	$23.0\cdot10^7$	$55.6\cdot10^7$
Tridecane	$1.48\cdot10^7$	$6.0\cdot10^7$	$22.5\cdot10^7$	$44.1\cdot10^7$
Dodecane	$1.34\cdot10^7$	$5.4\cdot10^7$	$21.3\cdot10^7$	$40.9\cdot10^7$
Undecane	$1.21\cdot10^7$	$4.8\cdot10^7$	$18.2\cdot10^7$	$37.4\cdot10^7$
Decane	$0.98\cdot10^7$	$3.9\cdot10^7$	$14.6\cdot10^7$	$28.6\cdot10^7$
Nonane	$0.74\cdot10^7$	$3.1\cdot10^7$	$11.7\cdot10^7$	$23.4\cdot10^7$
Toluene	$1.08\cdot10^7$	$4.0\cdot10^7$	$15.6\cdot10^7$	$23.2\cdot10^7$
Benzene	$1.04\cdot10^7$	$3.3\cdot10^7$	$7.3\cdot10^7$	$6.9\cdot10^7$

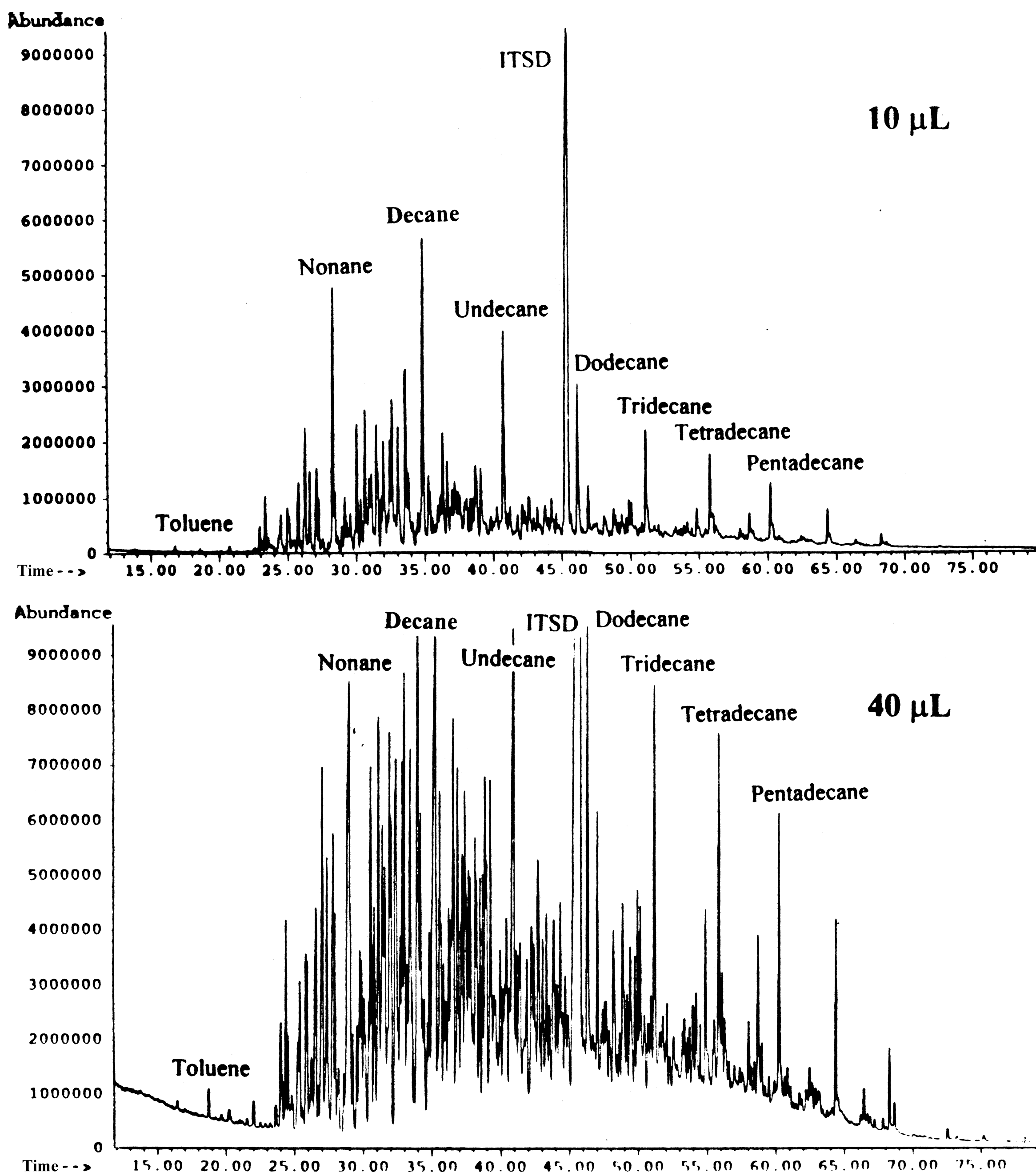

Fig. 1. Comparison of GC chromatograms of JP-8 (0.1%) in pentane; injection volume = 10 μl (top); injection volume = 40 μl (bottom).

During extraction, no significant emulsion was observed and the pentane layer was well separated from blood solution with the centrifuge. Fig. 2 shows example GC chromatograms of 0.016% JP-8 (Fig. 2B) and blank control (Fig. 2A) as extracted from blood–PBS. All target compounds were found and well separated. The chromatograms are essentially identical in qualitative character.

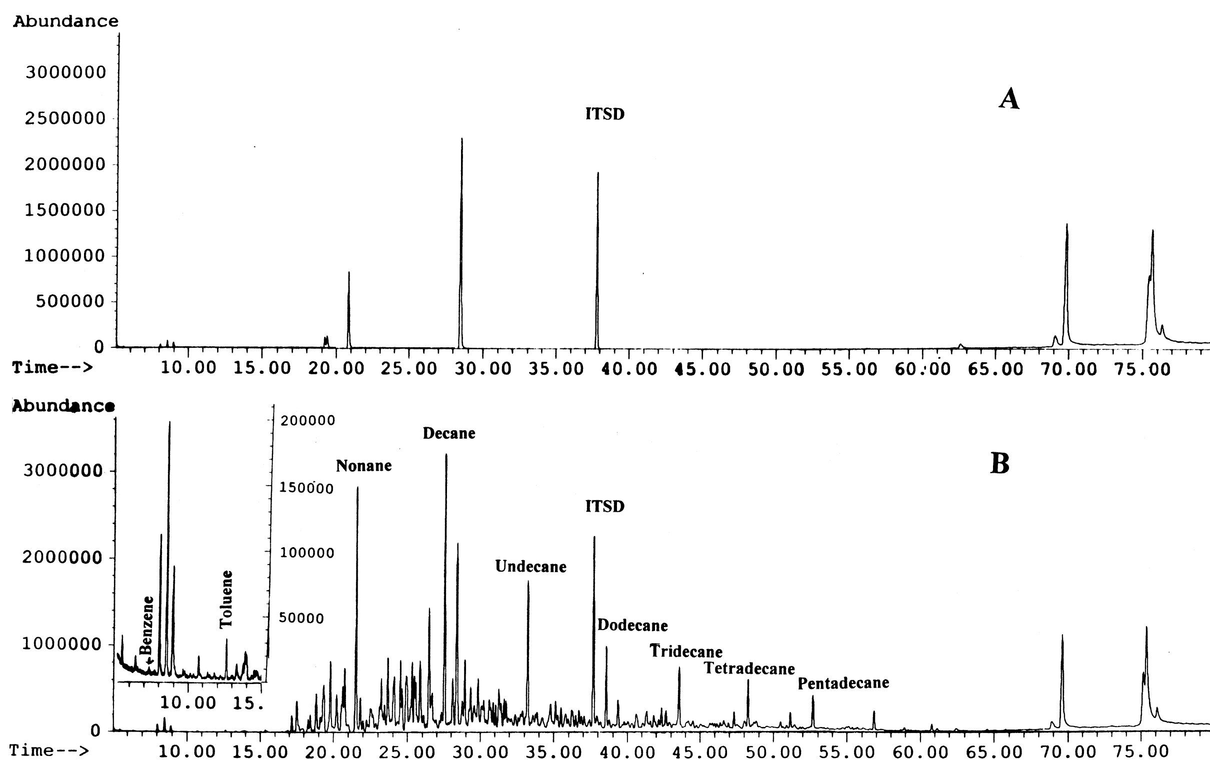

Fig. 2. GC chromatograms (full scan) of sample extracted from human whole blood spiked with JP-8 (B) and without JP-8 (A).

Table 4 shows the recovery efficiency of liquid–liquid extraction of representative JP-8 alkanes and aromatics from human blood. Besides the nine VOCs selected for the method development and optimization, six more aromatics including ethylbenzene, *o*-, *m*-, and *p*-xylenes, 1,3,5-trimethylbenzene as well as naphthalene were also analyzed and the corresponding recovery efficiencies were calculated at all concentrations except $C=0.00032\%$. The recovery efficiency for all the compounds analyzed ranged from 14.6 to 63.9%; apparent recovery efficiencies for aromatics were generally higher than for alkanes in part because the electron impact (EI) fragmentation pattern on the MS has a greater efficiency for making a small group of easily identifiable (unique) ions for aromatics. Alkanes tend to make many common fragments that are more likely impacted by interferants and background noise upon detection. Another potential reason is that the aromatics have greater aqueous solubility and are less likely to bind strongly to the lipids in the blood than the alkanes. However, we observed that when the concentration of JP-8 in blood–PBS solution was 0.0016% (v/v), neither toluene nor benzene could be detected on our GC–MS analytical system at the standard (1.5 μl) injection volume. We note that these two compounds are very minor constituents of JP-8 (area % of 0.0023 for benzene and 0.0319 for toluene compared to 4.88 for decane) and thus are not expected to appear at this level of JP-8 exposure.

To improve the detection limit, large-volume injection was then used to analyze the samples extracted from blood–PBS solution containing an even lower concentration of JP-8 (0.00032%, v/v). With 40 μl of sample injected, the monitored compounds (except benzene) were detected and the corresponding peak areas were recorded, as shown in the last column of Table 4.

Based on the standard curves, the detection limit of full scan GC–MS with molecular ion extraction for peak area measurement is about 0.06–0.6 ng on-column depending on the compound monitored.

Table 4
Recovery efficiency of liquid–liquid extraction of JP-8 from human blood

Compound	Recovery efficiency±S.E.[a] (%)			
	$C^{b}=0.016\%$ (1.5 μl injection)	$C=0.0032\%$ (1.5 μl injection)	$C=0.0016\%$ (1.5 μl injection)	$C=0.00032\%$ (40 μl injection)
Pentadecane	31.4±4.3	18.4±1.2	16.8±2.7	15.4±0.6
Tetradecane	32.4±3.7	14.6±0.7	17.2±2.7	21.6±4.5
Tridecane	33.5±3.0	19.1±0.4	18.8±2.9	22.2±3.9
Dodecane-d_{26} (I.S.)	100	100	100	100
Dodecane	30.9±0.0	21.6±0.2	22.4±4.1	31.0±6.5
Undecane	31.4±2.7	25.0±0.6	24.4±1.4	33.7±6.7
Decane	43.5±3.4	32.1±1.7	28.2±3.7	38.1±10.5
Nonane	49.7±3.6	37.1±2.3	30.5±5.4	40.2±5.8
Toluene	34.3±1.4	34.4±7.9	ND	45.1±13.4
Benzene	34.1±3.1	4.8±0.0	ND	ND
Ethylbenzene	62.2±16.5	30.5±2.5	52.0±5.9	NA
p+*m*-Xylene	62.1±15.9	27.1±2.8	49.2±6.1	NA
o-Xylene	61.7±15.9	28.0±3.7	55.1±6.6	NA
1,3,5-Trimethylbenzene	60.6±15.1	30.9±3.2	48.8±7.0	NA
Naphthalene	60.7±13.5	40.8±3.3	63.9±6.9	NA

[a] S.E.=Standard error.
[b] C=Concentration of JP-8 in blood–PBS solution.
ND=Measured but not detected, NA=not measured for this test.

If one extracts a 3-ml sample of blood (V_{blood}) and achieves a 200 μl final volume of solution after evaporation, we calculate the minimum original blood concentration (C_{blood}) of the compound required for achieving the minimum sample concentration (C_{inject}) for GC–MS analysis using:

$$C_{blood}\ (\text{ml/ml}) = \frac{C_{inject} \times 200\ \mu\text{l} \times 10^{-9}}{V_{blood} \times d_{voc} \times \text{R.E.}\ (\%)} \qquad (2)$$

where C_{inject}=minimum injection solution concentration (ng/μl) of the VOCs in JP-8 to achieve the GC–MS detection limit when 1.5 μl of sample is injected (ng/μl), d_{voc}=density (g/ml), V_{blood}= volume of blood (ml), R.E.=recovery efficiency and C_{blood}=minimum blood concentration of VOCs in JP-8 required to achieve the GC–MS detection limit when 1.5 μl of sample is injected.

As an example, we take nonane (d_{voc}=0.72 g/ml) for calculating C_{blood} by following Eq. (2) using these parameters: volume of blood (V_{blood}) is 3 ml, average recovery efficiency (R.E.) is 39.4% (calculated from the data in Table 4), and the minimum concentration of sample solution (C_{inject}) is 0.05 ng/μl. According to the standard curve, the minimum original blood concentration (C_{blood}) required for achieving the minimum sample concentration for GC–MS analysis will become $11.8 \cdot 10^{-9}$ ml per ml of blood, i.e., 11.8 ppbv. Table 5 lists the calculated C_{blood} of all VOCs monitored in JP-8; we find that these range from 8.2 to 168.9 ppbv for full scan MS.

In the previous study [28], it had been shown that using SIM acquisition for the MS detector could increase quantitative sensitivity 20-fold compared to using total ion acquisition (F_{SIM}=20). It would also lower the GC–MS detection limit and thus the minimum injection sample concentration (C_{inject}) by about 20-fold, i.e., $C^{\#}_{inject}=C_{inject}/F_{SIM}$, and thus the blood concentration of nonane, for example, to $0.59 \cdot 10^{-9}$ ml per ml of blood, i.e., 0.59 ppbv ($C^{\#}_{blood}=C_{blood}/F_{SIM}$). Furthermore, by using the large-volume injection technique as described in Section 3.4, the concentration of the analytes would be further lowered to about 33.3 fold (F_{LVI}) if 50 μl of sample is injected compared to 1.5 μl. This would bring the minimum blood concentration (C^{*}_{blood}) of nonane in JP-8 to $0.018 \cdot 10^{-9}$ ml per ml of blood, i.e., 0.018 ppbv or 18 pptv ($C^{*}_{blood}=C_{blood}/F_{SIM}F_{LVI}$). The minimum original blood concentrations for all monitored compounds required for achieving the detection limit for GC–MS analysis, with (C^{*}_{blood}) and without (C_{blood}) the consideration of using SIM acquisi-

Table 5
Calculated values for detection limit of the VOCs in blood for GC–MS analysis

Compound	R.E. average[a] (%)	C_{inject} (ng/μl)	C_{blood} (ml/ml)	F_{SIM}	F_{LVI}	C^*_{inject}[b] (ng/μl)	C^*_{blood}[c] (pptv)
Pentadecane	20.5	0.4	$168.9 \cdot 10^{-9}$	20	33.3	$6.0 \cdot 10^{-4}$	254
Tetradecane	21.5	0.4	$163.2 \cdot 10^{-9}$	20	33.3	$6.0 \cdot 10^{-4}$	245
Tridecane	23.4	0.4	$149.9 \cdot 10^{-9}$	20	33.3	$6.0 \cdot 10^{-4}$	225
Dodecane	26.5	0.2	$67.1 \cdot 10^{-9}$	20	33.3	$3.0 \cdot 10^{-4}$	101
Undecane	28.6	0.1	$31.5 \cdot 10^{-9}$	20	33.3	$1.5 \cdot 10^{-4}$	47
Decane	35.5	0.05	$12.9 \cdot 10^{-9}$	20	33.3	$0.75 \cdot 10^{-4}$	19
Nonane	39.4	0.05	$11.8 \cdot 10^{-9}$	20	33.3	$0.75 \cdot 10^{-4}$	18
Toluene	37.9	0.04	$8.2 \cdot 10^{-9}$	20	33.3	$0.61 \cdot 10^{-4}$	12
Benzene	19.5	0.04	$15.7 \cdot 10^{-9}$	20	6.7	$3.0 \cdot 10^{-4}$	117
Ethylbenzene	59.6	0.04	$4.5 \cdot 10^{-9}$	20	33.3	$0.61 \cdot 10^{-4}$	6.8
$p+m$-Xylene	52.1	0.04	$5.9 \cdot 10^{-9}$	20	33.3	$0.61 \cdot 10^{-4}$	8.9
o-Xylene	50.4	0.04	$6.1 \cdot 10^{-9}$	20	33.3	$0.61 \cdot 10^{-4}$	9.2
1,3,5-Trimethylbenzene	49.7	0.04	$5.4 \cdot 10^{-9}$	20	33.3	$0.61 \cdot 10^{-4}$	8.1
Naphthalene	60.2	0.04	$4.4 \cdot 10^{-9}$	20	33.3	$0.61 \cdot 10^{-4}$	6.6

[a] Data is the average R.E. at different concentrations in Table 4.
[b] $C^*_{inject} = C_{inject}/F_{SIM}F_{LVI}$.
[c] $C^*_{blood} = C_{blood}/F_{SIM}F_{LVI}$.

tion for the MS detector and large-volume on-column injection technique, are calculated and listed in Table 5. The blood concentrations at the detection limit for GC–MS analysis are generally within ppt level, ranging from 6.6 to 254 pptv depending on each individual compound monitored. Benzene's volatility with respect to the pentane solvent makes it a special case. When the injection volume is larger than 10 μl, the proportional increase of peak area could not be observed and therefore $F_{LVI} = 6.7$ is used for calculating C^*_{blood} for benzene in Table 5. The changes in ultimate sensitivity for the n-alkanes with respect to molecular mass is incidental; it is an effect of the respective sensitivity characteristics of our MS detector coupled with the behavior of our particular GC column and temperature program.

3.6. Optimized method for extraction of JP-8 from human whole blood

The following text consolidates the results of this work to a simple and optimized procedure. Specifically, the resulting optimized procedure for measuring the blood concentration of VOCs from JP-8 exposure has the following steps as practiced in our laboratory:

1. Collect blood sample (3–7 ml) in a 28-ml glass centrifuge tube containing heparin.
2. Dilute the blood sample with sodium-chloride-saturated PBS solution (1:1).
3. Extract the blood–PBS solution with pentane (1:1) containing internal standard benzene-d_6 and pentadecane-d_{32} for 20 min by gentle hand shaking or with an electronic shaker.
4. Centrifuge the mixture at 1600 g for 30 min.
5. Separate the pentane layer from the blood; discard blood remnants; seal pentane extract in separate vial; transport to laboratory on dry ice.
6. Dry pentane extract over anhydrous sodium sulfate; concentrate the solution in a K–D evaporator to 200 μl.
7. Add dodecane-d_{26} (2000 ng/μl, 10 μl) as internal analytical standard for analysis.
8. Analyze by GC–MS and large-volume injection; use a 40- or 10-μl injection depending on the need for benzene quantitation.
9. Calculate the original blood concentration of the VOCs in JP-8 using Eqs. (1) and (2).

Some of the above steps could be changed without affecting the eventual outcome. For instance, step 1 could employ standard Vacutainer tubes to collect blood with subsequent transfer to glass centrifuge

vials; step 2 could allow addition of water and salt separately; and the use of internal standards benzene-d_6 and pentadecane-d_{32} in step 3 is a quality assurance precaution for eventual shipping of remotely collected samples. Finally, the ''gentle hand shaking'' method for mixing blood and pentane could be replaced with a mechanical vortexing method as long as care is taken to assess any changes in recovery efficiency.

4. Conclusion

In this study, a method for analyzing fresh whole blood for JP-8 jet fuel exposure has been optimized in both extraction and GC–MS analysis techniques to achieve ppt level sensitivity.

Given that VOCs typically exhibit blood/breath ratios ranging from about 2.5 to 55 [35], and that control group breath samples levels are typically in the low- or sub-ppbv range [28], we conclude that the blood method here is more than sufficiently sensitive for both environmental and occupational exposure assessment to JP-8.

The major accomplishment here is one of practicality. All extraction and sample processing equipment used is standard biological laboratory instrumentation, all reagents are simple and readily available, and the GC–MS method is implemented on a very modest bench top system with only a minor modification to the arrangement of the analytical columns. Finally, the method is deliberately designed to extract and stabilize the analytes in the field. This minimizes losses and reduces the chances of bringing potentially infectious materials to the laboratory; we do, however, treat the extracts as potentially infectious under universal precaution protocols.

We anticipate that the application of this methodology to real blood samples in the field will demonstrate unambiguous measurement of blood-borne chemicals from common human exposures, including low-level incidental environmental exposure. We expect that defensible quantitative links can be forged between blood levels and exhaled breath concentrations to eventually allow non-invasive breath measurements to be used for larger population-based studies.

Though this method has been developed using JP-8 jet fuel as the target pollutant, we anticipate applying it also to polyaromatic hydrocarbon (PAH) exposure and to common pesticides exposure scenarios.

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