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Method for liquid–liquid extraction of blood surrogates for assessing human exposure to jet fuel

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Abstract

A baseline method of liquid–liquid extraction for assessing human exposure to JP-8 jet fuel was established by extracting several representative compounds ranging from very volatile to semi-volatile organic compounds, including benzene, toluene, nonane, decane, undecane, tridecane, tetradecane and pentadecane, from PBS buffer. Some specific techniques for solvent selection, solvent evaporation, and GC analysis were developed to accommodate this wide range of constituents of JP-8. The application of the established method to the extraction and quantitative analysis of JP-8 from PBS and bovine plasma was demonstrated. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: JP-8 jet fuel; Volatile organic compounds

1. Introduction

Protection of human health from environmental pollutants begins with the accurate assessment of the actual exposure. Such information is used to plan further health-related research and to serve as a baseline to confirm the efficacy of remedial actions. To this end, we have developed a method for determining the extent of human exposure to JP-8 jet fuel, which is considered the most common chemical exposure in the military. It is the fuel for all land-based internal combustion engines including trucks, jeeps, tanks, and generators; all aircraft except for some helicopters; and cooking and heating for forward-deployed forces. Additionally, JP-8 is essentially identical to Jet A-1, the commercial aircraft aviation fuel. The U.S. Air Force Human Systems Center at Brooks Air Force Base, San Antonio, TX, has taken the science lead in investigating the extent and effects of human exposure to JP-8.

JP-8 is a kerosene-based jet fuel that has recently replaced JP-4 because of its higher flash point and comparatively lower vapor pressure; this reduces evaporative losses and resists crash-induced fires and explosion [1]. Like other petroleum distillate fuels, JP-8 is a complex mixture of aromatic and aliphatic hydrocarbons [1]. Although JP-8 has been considered relatively nontoxic [2], several recent studies have indicated the toxic effect of JP-8 on the immune system [3], postural balance [4], pulmonary function [5], and embryo growth [6]. Therefore, occupational exposure to JP-8 in the Air Force has now become a real concern in terms of long-term

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health consequences, particularly for flight and ground crew personnel during preflight operations and maintenance and for personnel performing routine tasks. Personal exposure at an air force base occurs as occupational exposure for those involved with fuel and aircraft handling and as incidental exposure for all personnel, regardless of career field, primarily through inhalation of ambient fuel vapors and exhaust. Similar exposures are expected for the commercial aviation sector, including the traveling public.

Exposure assessment is generally performed with ambient measurements where one collects and analyzes samples of air, water, food, soil, etc., and then calculates the overall human impact from these sources. Since breath is considered to be the largest pathway for removing volatile organic compounds (VOCs) from the body [7], various attempts have been made to relate breath levels to known exposures [7–10]. Over the past three years, we have developed sampling and analysis technology to directly assess the VOC content of individual, alveolar, exhaled breaths. This is an unambiguous measure of exposure to exogenous chemicals, and because the breath concentration reflects the blood concentration, this measurement technology gives a direct, individual exposure assessment for all routes of exposure. Through collection of a series of breath samples during and after exposures, researchers have developed data interpretation techniques that allow estimation of biological parameters that indicate chemical distribution in the body, residence times in bodily compartments (such as blood, highly perfused tissues, and lesser perfused tissues), and overall capacity or dose [11–16]. With this technique, breath samples from various groups of air force personnel were collected. The JP-8 exposure of all subjects was determined, ranging from slight elevations compared to the control cohort to more than 100 times the control values [17].

The major point of concern for using breath as a surrogate for blood and tissues measurement is that the relationships are often not well understood. The living organism is a complex entity, but much of the published literature of the partition coefficients is based upon in vitro measurements; the few studies available that link human blood and breath VOC concentrations directly have shown that in the steady state, this is an adequate relationship. So far, however, none have properly reflected the extremely rapid changes at the time interface between exposure and elimination. Therefore, a direct blood measurement is the key to linking environmental factors, exposure and risk. Additionally, blood-borne dose and exhaled breath measurements have not, as yet, been directly and quantitatively linked for JP-8 fuel.

Measurement of trace amounts of toxic chemicals from blood is a challenging task. Measurements of VOC levels in blood are reported in earlier studies [18–24]. Ashley et al. [25,26] have improved the method recently to determine VOCs in human blood from a large sample population by using purge and trap gas chromatography–mass spectrometry (GC–MS) and standard blood collection techniques. The results of these validity studies indicate detection limits in the low part-per-trillion range for most analytes analyzed [25,26]. However, blood is a highly complex matrix with numerous compounds present that are separated along with VOCs when purge-and-trap or headspace sampling is used. These additional compounds can interfere with the analytical response of the VOCs of interest and prevent accurate characterization [26]. In addition, as mentioned earlier, JP-8 is also a complex mixture, containing numerous aromatic and aliphatic hydrocarbons with a wide range of physical and chemical properties, such as boiling point and vapor pressure [1]. Therefore, applying this technique to measure JP-8 from blood presents problems such as interferences from the purge and trap of water and other polar VOCs. Also, non-polar analytes such as the lighter hydrocarbons tend to partition out of the aqueous blood and become lost in headspace. Thus, sample integrity becomes an issue during prolonged transport and storage times, and achieving accurate analytical results generally requires the use of isotopically labeled surrogates for all compounds of interest to correct for losses.

Recently, a new method called solid-phase microextraction (SPME) has been developed, wherein the fluid headspace is collected with a fused-silica fiber coated with a special stationary phase, retracted into a syringe needle sheath, and then directly injected onto a gas chromatograph [27]. This is an elegant method, especially well suited for the more volatile of the analytes; however, it requires special analytical tools, is most likely poorly matched for C_{12} and higher hydrocarbons, and requires that a fresh

(or preserved) whole-blood sample be present in the laboratory.

Liquid–liquid extraction is the fundamental technique for the separation of a chemical species from a medium or from other coexisting components [28]. Particularly for partial purification of a biological fluid such as blood containing toxic chemicals, the most widely used procedure is extraction into an organic solvent [29]. This type of method is preferable for us because it allows in-field stabilization of the analytes into a compatible solvent prior to shipping and storage, and because it avoids having to routinely bring potentially infectious materials (whole blood) into the laboratory. The major issue for this type of technique is choosing an appropriate solvent for all the compounds of interest that preferentially partitions them from the blood, can be easily stored and transported without risk of evaporative loss, and yet can be differentially reduced in volume to allow preconcentration for sensitive analysis.

In this paper, we present the development of a liquid–liquid extraction method for assessing human exposure to jet fuel JP-8. For this method, we used a phosphate-buffered saline (PBS) solution as a blood surrogate and developed the techniques for extraction, solvent evaporation and GC–MS analysis of representative VOCs in JP-8, including benzene, toluene, nonane, decane, undecane, tridecane, tetradecane and pentadecane. The standardized procedure thus established was then applied to the liquid–liquid extraction of JP-8 sample from blood surrogate PBS solution. We explored the use of two solvents–pentane and dichloromethane (DCM), different solvent volume reduction techniques, and different GC injection techniques. Finally, by extracting JP-8 from spiked bovine plasma, we demonstrated that this method is likely applicable to complex biological media. This is to be considered a baseline method from which more specific or tailored methods can be developed for various practical applications.

2. Experimental

2.1. Materials

Solvents such as pentane, DCM and acetone were purchased from Burdick & Jackson Co. (Muskegon, MI, USA) as GC–MS grade. Reagents such as benzene, toluene, nonane, decane, undecane, dodecane, tridecane, tetradecane and pentadecane were purchased from PolyScience (Niles, IL, USA) as analytical standards. Deuterated dodecane (dodecane-d_{26}) was purchased from Cambridge Isotope Laboratories, Inc. (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). 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 surrogate spikes, the primary jet fuel sample was collected directly from the fuels facility at Robins Air Force Base, Warner-Robins, GA, with a 1-liter evacuated SilcoCan (Restek Corp., 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 canister with essentially zero vapor headspace. Bovine plasma for the final demonstration test was obtained from Pel Freez (Rogers, AR, USA).

2.2. GC–MS apparatus and condition

Chromatographic separation was achieved with an HP 5890 Series II gas chromatograph (Hewlett Packard, Santa Clarita, CA, USA) directly connected to the HP 5971A mass selective detector. The system was controlled by an MS Chemstation (Windows 3.1). The separation was made with a fused-silica capillary column, 30 m long with an internal diameter of 0.25 mm, coated with 100% dimethyl polysiloxane (1.0 μM thickness, Rtx-1, Restek Corporation, Bellefonte, PA, USA). A guard column (10 M×0.32 mm I.D., Alltech Associates, Deerfield, IL, USA) was used. The following oven temperature program was used during the analysis: 35°C (pentane) or 40°C (DCM)×5 min and then 3°C min^{-1} to 200°C (VOCs) or 300°C (JP-8). About 1.5 μl of sample was directly injected on the column. The injector temperature was 35°C for the samples in pentane and 40°C for the samples in DCM. Helium

was used as the carrier gas (inlet pressure, 100 kPa). The MS conditions were as follows: the temperature was 250°C; the instrument was operated in full-scan mode (44 to 350 amu) with electron impact ionization; parameter values were optimized for maximum sensitivity. For some diagnostic comparisons, samples were analyzed by using the selective ion monitoring (SIM) mode, where we acquired molecular ions and base peak ions for the selected compounds of interest.

2.3. Procedure for the assessment of recovery efficiency from solvent evaporation

The evaporation solutions were prepared by dissolving benzene, toluene, nonane, decane, undecane, tridecane, tetradecane and pentadecane in acetone and diluting the solutions to concentrations of 100 ng μl^{-1}, 10 ng μl^{-1} and 1 ng μl^{-1}. Blank solvent was used as a control. Next, 200 μl of each solution was mixed with 18 ml of pentane or DCM. The concentrations of the evaporation solutions were then at 1.1 ng μl^{-1}, 0.11 ng μl^{-1} and 0.011 ng μl^{-1}, respectively. The solution was evaporated by either streaming N_2 through the solution at room temperature or using a Kuderna–Danish (K–D) evaporation concentrator in a water bath of 40–45°C for pentane or 45–50°C for DCM in the presence of boiling chips. The evaporation process was stopped when 200 μl of solution was left.

2.4. Procedure for the assessment of recovery efficiency from the extraction–evaporation process

Similarly, the extraction solutions were prepared by dissolving benzene, toluene, nonane, decane, undecane, tridecane, tetradecane and pentadecane in acetone and diluting to concentrations of 100 ng μl^{-1}, 10 ng μl^{-1} and 1 ng μl^{-1}; blank solvent was used as a control. First, 200 μl of each of these solutions was mixed with 6 ml PBS buffer, resulting in spiked surrogate blood samples with 3.23 ng μl^{-1}, 0.323 ng μl^{-1} and 0.0323 ng μl^{-1}, respectively, of each analyte. These surrogate samples were then extracted with 6 ml of pentane or DCM three times by vortexing the solution for 1 min. The organic phases (pentane or DCM) were then separated from the aqueous buffer and dried over anhydrous sodium sulfate. After separation from the drying agent, the remaining solutions were evaporated following the N_2 stream or Kuderna–Danish procedures described above. The evaporation process was stopped when 200 μl of solution was left.

2.5. Procedure for the assessment of the recovery efficiency of JP-8 from liquid–liquid extraction

The JP-8 solutions were prepared by dissolving JP-8 in acetone and diluting to concentrations of 5%, 1% and 0.5%; blank solvent was used as the control. First, 200 μl of each solution was mixed with 6 ml PBS buffer, and the concentration of JP-8 in PBS solution was thus diluted to 0.16%, 0.032% and 0.016%, respectively. These were then treated as above and eventually reduced to 200-μl extracts. For demonstration purposes, we repeated this procedure for a subset of samples and blanks using bovine plasma as a blood surrogate. The plasma solution (6 ml) was first treated with 100 μl of concentrated sulfuric acid in ice and then extracted with pentane (18 ml). The mixture was centrifuged at 1600 $g \times 10$ min. The organic phase was treated as above until 200 μl of extract was obtained.

2.6. GC–MS analysis of the samples and calculation of the recovery efficiency

The VOC solutions of 100 ng μl^{-1}, 10 ng μl^{-1} and 1 ng μl^{-1} or JP-8 solutions of 5%, 1% and 0.5% in pentane or DCM were used as control solutions. The samples were immediately stored in a refrigerator after their preparation. Just before GC–MS analysis, each sample was mixed with 10 μl of an internal standard solution at 2000 ng μl^{-1} dodecane. An aliquot of these samples (1.5 μl) was injected directly onto the GC column, at 35°C for a pentane solution and 40°C for a DCM solution. Two or three analyses were performed for each sample. The relative area to internal standard for each compound in sample solution was compared with the relative area to internal standard for each compound in the control solution of the same concentration. The percentage of the recovery was thus calculated as follows.

$$\text{Recovery\%} = \frac{(VOC_s/IS_s)}{(VOC_c/IS_c)} \times 100\% \quad (1)$$

where VOC_s = peak area for sample VOC; IS_S = peak area for sample internal standard; VOC_C = peak area for control VOC; IS_C = peak area for control internal standard.

Generally, this procedure was followed for all quantitative samples, controls, tests and calibrations to ensure internal consistency. For most of this work we used full-scan analysis; for some demonstrations, however, we employed specific SIM protocols as mentioned above.

2.7. Generation of standard curves

Peak-area ratios obtained by analyzing the target VOCs in duplicate with known concentrations (0.1, 0.4, 0.7, 1.0, 4.0, 7.0 and 10.0 ng μl^{-1}) were used to construct the standard curves. The equations generated for each compound (Table 6) were used to calculate the concentration of injection solutions.

3. Results and discussion

JP-8, like other petroleum distillate fuel, is a complex mixture of aromatic and aliphatic hydrocarbons [1]. Fig. 1 shows a typical GC chromatogram of a JP-8 sample collected from Air Force jet fuel at Robins AFB. The labeled compounds were identified by a GC–MS detector. We chose C_9–C_{15} alkanes, benzene and toluene as our monitoring targets for JP-8, because as can be seen in Fig. 1, straight-chain hydrocarbons C_9–C_{15} are the major components in the JP-8 fuel, and benzene and toluene have long been considered to have important effects on human health.

3.1. Choice of solvent

The ideal solvent for liquid–liquid extraction should be (i) clean and easily recoverable, (ii) non-toxic and not highly flammable, (iii) immiscible with water, (iv) of suitable volatility, (v) of high chemical stability and inertness, (vi) not prone to form an emulsion, and most important, (vii) able to selective-

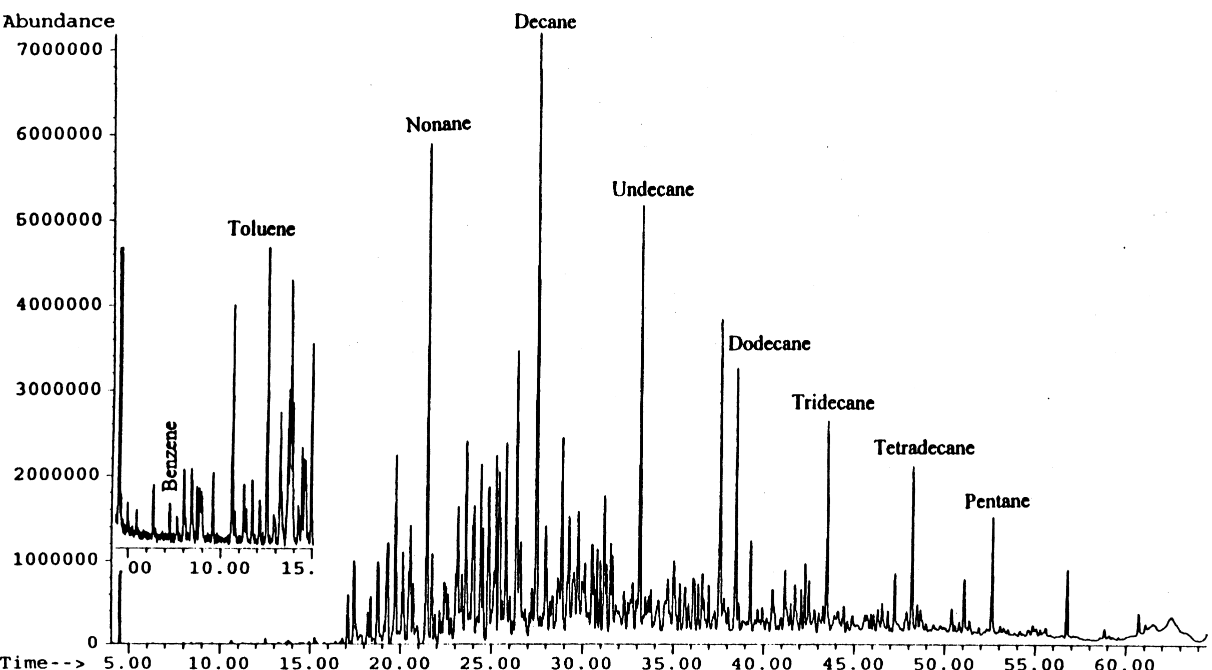


Fig. 1. Chromatogram of JP-8 sample collected from U.S. Air Force. The concentration is 0.5% in pentane. The identification of the selected peaks in the following table was conducted by using the Wiley library program.

ly dissolve the compounds to be extracted to achieve a high yield of extraction [30]. Because JP-8 consists of a mixture of aromatic and aliphatic hydrocarbons, many of which are VOCs, a desirable extraction solvent should have a lower boiling point and higher vapor pressure than the compounds to be extracted to minimize loss of the VOCs during the evaporation process. Table 1 lists two important physical properties, boiling point and vapor pressure, for each compound and solvent used in our study. Pentane and DCM were first chosen as the extracting solvents because (i) chemically, pentane itself is a straight-chain alkane like the major components in JP-8 and (ii) physically, it has low boiling point of 36°C and vapor pressure of 159 mmHg at 25°C (Table 1); similarly, DCM has a low boiling point (40°C) and relatively high vapor pressure (58.2 mmHg). In addition, DCM is a good solvent for the relatively more polar aromatics such as benzene and toluene, and it is non-flammable.

3.2. Development of analytical techniques and conditions for quantitative GC–MS analysis

As mentioned earlier, JP-8 is a mixture of hundreds of aliphatic alkanes and aromatic compounds. With the GC oven temperature program described in the experimental section, the compounds to be monitored were well separated (Fig. 1). However, during our early quantitative GC–MS analysis of the liquid–liquid extraction samples, we noticed that the relative peak areas of some compounds fluctuated greatly for different injections of the same sample solution. We then conducted a series of experiments examining what factors affected the quantitative outcome of GC–MS analysis. We found that the fluctuation was largely dependent on injection technique, solvent choice, and injection temperature.

Standard methods for manual injection suggest, after inserting the syringe needle into the injector, to 'wait for 2 s for the needle to get hot ... then evenly, over a period of about 1 s, inject the contents of the syringe into the injector. Wait for an additional 2 s, then withdraw the needle from the injector' [31]. However, from our experiments we found that because of the wide range of analyte volatilities and the compromise conditions of temperature as dictated by the solvent, the syringe needle must be withdrawn from the injection port immediately after injection. Otherwise, significant reduction of relative peak area will result, especially for the compounds with low boiling points and high vapor pressure (similar to the solvent). Fig. 2 shows the comparison of relative peak areas of the compounds analyzed from the samples by the two different injection techniques. An interesting observation was that a smaller effect was seen on the samples in DCM and acetone solution (not shown). To accommodate all analytes of interest in a single injection and analysis, we chose to use this rapid withdrawal method.

The effect of the injection temperature on the quantitative GC analysis was also solvent dependent. Because the sample is injected in a 'liquid' state directly on the column, on-column injection depends

Table 1
Physical properties of the VOCs and solvents used in the study

VOCs and solvents	Molecular formula	Molecular weight	Boiling point (°C)	Vapor pressure at 25°C (mmHg)
n-Nonane	C_9H_{20}	128	151	4.45
n-Decane	$C_{10}H_{22}$	142	174	1.43
n-Undecane	$C_{11}H_{24}$	156	196	4.12E−01
n-Dodecane	$C_{12}H_{26}$	170	216	1.36E−01
n-Tridecane	$C_{13}H_{28}$	184	234	5.58E−02
n-Tetradecane	$C_{14}H_{30}$	198	252	1.16E−02
n-Pentadecane	$C_{15}H_{32}$	212	270	3.43E−03
Benzene	C_6H_6	78	80	95.20
Toluene	C_7H_8	92	110.6	28.40
CH_2Cl_2	CH_2Cl_2	84	40	58.2
Pentane	C_5H_{12}	72	36	159

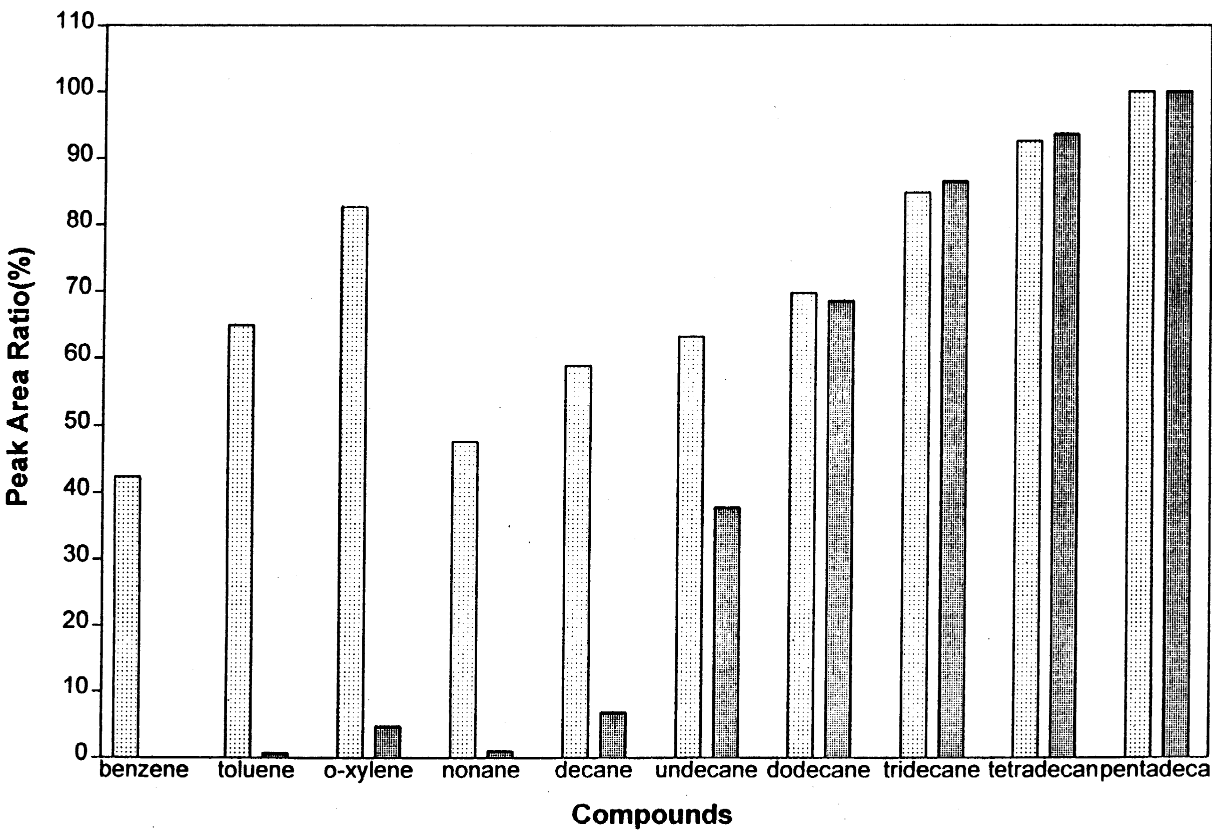


Fig. 2. Effect of injection technique in pentane solution at 35°C. The detailed experimental conditions are described in the experimental section. ▭ needle withdrawn immediately, ▬ needle withdrawn after 2 s.

either on a cold trapping of the sample or a 'solvent effect' to concentrate the sample at the head of the column. Consequently, for optimal results, the sample is injected at a temperature near the boiling point of the solvent. For example, the recommended injection temperature for pentane is 30–45°C and for DCM is 35–50°C [32]. Again from our experiments, we determined that when the compounds were in pentane solution, the relative peak area was decreased by up to 50% for JP-8 constituent compounds with low boiling points and high vapor pressure when injected at 40°C in comparison to when they were injected at 35°C and 25°C. This result indicates when pentane is used as sample solvent, the injection temperature must be at or lower than the boiling point of the analyte, especially when compounds with a low boiling point and a high vapor pressure are analyzed. This effect was not as significant when the samples were in DCM or acetone. Thus, injection temperature was critical because of the experimental constraints and the physical properties of JP-8: we needed to accommodate a wide range of volatilities with only a few choices of solvent.

3.3. Technical development for efficient solvent evaporation

In testing methods for measuring compounds at the limit of sensitivity, it is necessary to evaporate the solvent so that the compounds can be reconstituted in small volumes for quantitative and qualitative analysis. We first attempted to concentrate the JP-8 VOCs in a pentane solution by flowing a pure N_2 stream through the solution until the desired volume was reached. Experimentally, 200 μl of the

solution containing the VOCs at 100 ng/μl (200 μl) was mixed with 18 ml of pentane. N_2 gas was bubbled through the solution at room temperature until 200 μl of the solution was left. Each compound in both the evaporated solution and the control solution was then analyzed qualitatively and quantitatively by using GC–MS in the presence of the internal standard dodecane (100 ng μl^{-1}). The recovery percentage, calculated as in Eq. (1), varied according to the compound; however, a recovery efficiency of only 1.7% to 29.1% was obtained (Table 2). To improve the recovery efficiency from the solvent evaporation process, a Kuderna–Danish (K–D) concentrator was used to selectively evaporate the solvent. The K–D concentrator was operated with the sample in a water bath at a temperature 5–10°C higher than the boiling point of the solvent to be evaporated; the pentane solution was gently refluxed at 40–45°C and the DCM solution at 30–35°C. The solvent was gradually evaporated and the VOCs were concentrated until about 200 μl of the solution was left. The recovery efficiency of VOCs in pentane solution from K–D evaporation, for example, ranged from 11.1% to 46.1% (Table 2). A 1.6- to 6.5-fold improvement in recovery efficiency over the N_2 flow technique was achieved by using the K–D evaporator, depending on the compound.

3.4. Recovery efficiency from solvent evaporation and from liquid–liquid extraction–evaporation

Liquid–liquid extraction includes two separate procedures, extraction and evaporation. To better understand the contribution to the recovery efficiency from each procedure, we examined the recovery efficiency of each step. We assessed the recovery efficiency from both evaporation (E) and extraction–evaporation (EE) of pentane solutions and DCM solutions by following the procedure described above in the experimental section and performing the calculations described in Eq. (1).

From evaporation alone, recovery efficiencies ranging from 11.7±1.8% to 46.8±2.1%, 27.3±15.4% to 46.7±8.8%, and 12.8±1.6% to 31.5±6.4% were obtained with pentane as solvent (Table 3), and from 26.4±1.3% to 53.0±8.1%, 31.6±7.7% to 53.1±6.3%, and 32.7±4.5% to 51.0±4.8% with DCM as solvent (Table 4), at concentrations of 1.1 ng μl^{-1}, 0.11 ng μl^{-1}, and 0.011 ng μl^{-1} in evaporation solution, respectively. We accomplished the extraction–evaporation of VOCs from PBS solution with pentane and with DCM as solvent by extracting VOCs in PBS solution (3.23 ng μl^{-1}, 0.323 ng μl^{-1}, and 0.0323 ng μl^{-1}) with solvent (6 ml) three times and then evaporating the solvent in the K–D evaporator as described earlier. The recovery efficiency from the extraction–evaporation process is also summarized in Tables 3 and 4. Table 3 shows that recovery efficiencies with pentane as solvent ranging from 7.1±0.4% to 59.0±7.8%, 8.1±1.5% to 47.8±0.9%, and 9.2±1.0% to 32.5±1.9% were obtained at concentrations of extraction solution of 3.3 ng μl^{-1}, 0.33 ng μl^{-1}, and 0.033 ng μl^{-1}, respectively. Similarly, the data from the DCM solution (Table 4) showed the recovery efficiency of liquid–liquid extraction

Table 2
Comparison of recovery efficiency by the two solvent evaporation methods

VOC	Recovery% from N_2 evaporation (1.11 ng μl^{-1})	Recovery% from K–D evaporation (1.11 ng μl^{-1})	Recovery% (K–D)/Recovery% (N_2)
Pentadecane	29.1	46.8	1.6
Tetradecane	25.1	43.3	1.7
Tridecane	16.8	40.9	2.4
Dodecane	100	100	100
Undecane	9.8	38.1	3.9
Decane	9.3	37.5	4.0
Nonane	9.4	37.6	4.0
Toluene	7.9	27.1	3.4
Benzene	1.7	11.1	6.5

Table 3
Recovery efficiency of evaporation and extraction–evaporation of VOCs from pentane solution[a]

Compound	Recovery efficiency (%)±S.E.					
	E 1.11 ng μl^{-1}	EE 3.32 ng μl^{-1}	E 0.11 ng μl^{-1}	EE 0.33 ng μl^{-1}	E 0.011 ng μl^{-1}	E 0.033 ng μl^{-1}
Pentadecane	46.8±2.1	59.0±7.8	46.7 ’ 8.8	47.8±0.9	31.5±6.4	32.5 ’1.9
Tetradecane	43.3±2.6	54.4±7.6	41.3±2.8	43.5±0.8	31.6±0.2	32.5±0.2
Tridecane	40.9±2.0	48.5±5.2	51.0±13.4	41.7±1.2	29.3	33.3±2.1
Dodecane (ITSD)	100	100	100	100	100	100
Undecane	38.1±2.5	39.0±4.3	35.4±4.2	35.7±2.1	28.9±4.4	25.9±3.4
Decane	37.5±2.8	36.2±4.0	35.8±0.3	31.1±2.0	28.3±3.8	24.1±2.8
Nonane	37.6±4.9	31.4±2.4	38.4±1.1	29.5±1.3	33.9±1.0	30.0±2.0
Toluene	27.1±5.3	21.7±2.3	28.9±1.9	23.8±0.6	32.5±2.1	35.9±3.3
Benzene	11.7±1.8	7.1±0.4	27.3±15.4	8.1±1.5	12.8±1.6	9.2±1.0

[a] Abbreviations: S.E.: standard error, E: evaporation, EE: extraction–evaporation, ITSD: internal standard.

ranged from 18.3±2.2% to 47.7±6.3%, 33.0±5.4% to 47.0±6.6%, and 18.6±2.2% to 44.6±6.3% at corresponding concentrations.

From Tables 3 and 4, it can be seen that in general, aliphatic alkanes, especially those with high carbon numbers, tend to be recovered with higher efficiency in pentane solution than in DCM solution. In contrast, aromatics, especially benzene, were recovered from the extraction–evaporation process with higher efficiency in DCM solution than in pentane solution.

In addition, we can see that in both solvents, the recovery efficiencies from both evaporation alone and from the extraction–evaporation process vary in a significant range, which seemed to largely depend on the compound and the concentration of the compounds in the solution. As expected, the compounds with higher boiling points and lower vapor pressure (Table 1) tended to be recovered with higher efficiency. The difference in recovery efficiency caused by the difference in the concentration of the compounds was believed to result from systematic losses during the transformation of reagents and solutions throughout the process.

In a final comparison of the recovery efficiency of these two processes–evaporation alone and extraction–evaporation, the decrease in recovery from the liquid–liquid extraction process appears to occur

Table 4
Recovery efficiency of evaporation and extraction–evaporation of VOCs from DCM solution[a]

Compound	Recovery efficiency (%)±S.E.					
	E 1.11 ng μl^{-1}	EE 3.32 ng μl^{-1}	E 0.11 ng μl^{-1}	EE 0.33 ng μl^{-1}	E 0.011 ng μl^{-1}	EE 0.033 ng μl^{-1}
Pentadecane	53.0±8.1	47.7±3.0	53.1±6.3	47.0±6.6	51.0±4.8	44.6±6.3
Tetradecane	52.8±7.6	46.5±2.8	58.3±0.9	38.8±3.6	43.6±8.9	36.2±2.3
Tridecane	55.3±9.9	44.3±2.3	48.1±7.5	37.9±2.9	44.1±6.3	36.8±3.1
Dodecane (ITSD)	100	100	100	100	100	100
Undecane	51.6±6.2	40.1±3.0	44.0±6.5	33.8±2.4	51.4±2.9	27.9±3.1
Decane	50.5±6.3	38.52±3.8	47.6±1.7	30.9±2.7	45.1±4.2	25.3±2.6
Nonane	47.8±4.9	33.4±3.5	42.9±1.5	27.4±1.9	52.2±6.5	32.1±1.6
Toluene	44.6±4.1	33.1±3.2	41.5±2.2	31.0±2.8	63.1±8.8	41.2±0.7
Benzene	26.4±1.3	18.3±2.2	31.6±7.7	33.0±5.4	32.7±4.5	18.6±2.2

[a] Abbreviations: DCM: dichloromethane, S.E.: standard error, E: evaporation, EE: extraction–evaporation, ITSD internal standard.

mainly during the solvent evaporation step. The recovery efficiency from extraction ranged from 56% to more than 100%.

3.5. Recovery efficiency from liquid–liquid extraction of JP-8

For the liquid–liquid extraction of JP-8, we followed a procedure similar to that described for liquid–liquid extraction of VOCs. JP-8 solution at different concentrations (0.16%, 0.032%, and 0.016%) in blood surrogate PBS solution was extracted with pentane or DCM, evaporated in the K–D concentrator, and analyzed quantitatively through GC–MS. Instead of regular dodecane, deuterated dodecane (dodecane-d_{26}) was used as the internal standard for quantitative analysis. The recovery efficiency of JP-8 from liquid–liquid extraction was calculated, again by following Eq. (1), and the data are shown in Table 5. Following a trend similar to that seen for the liquid–liquid extraction of VOCs, the recovery efficiency of JP-8 liquid–liquid extraction varied with the extraction solvent used, the concentration of JP-8 in PBS solution, and the physical and chemical properties of the compounds analyzed. With pentane as solvent, the higher the concentration of JP-8 in the solution was, the better the recovery efficiency. At the same concentration, the recovery efficiency of aliphatic compounds is better than that for the aromatics, especially for benzene. With DCM instead of pentane as solvent, a relatively lower recovery efficiency for the long-chain aliphatics and higher recovery efficiency for the relatively short-chain aliphatics and aromatics was shown.

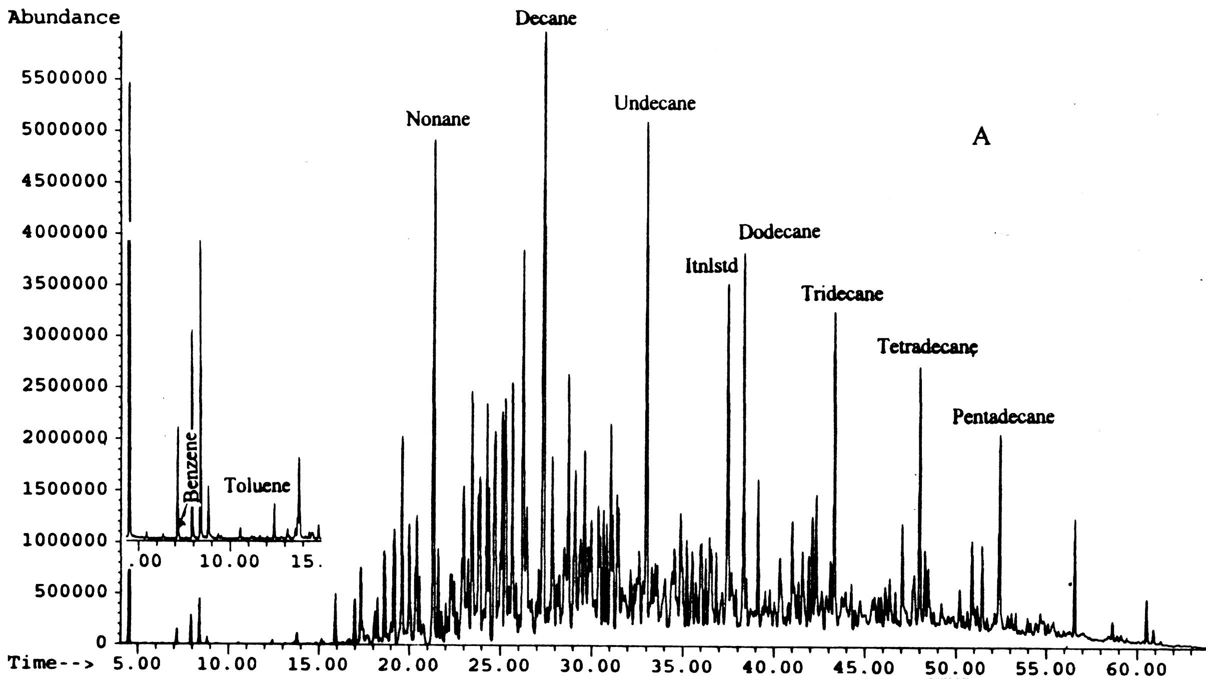
3.6. Application of the established method to the extraction of JP-8 from bovine plasma

Although the bulk of the methods development work was performed by using PBS as the blood surrogate for safety and convenience, a limited number of samples were tested with bovine plasma, which is a much more complex matrix. A detailed evaluation of plasma extraction is beyond the scope of this paper; however, in Fig. 3, a set of chromatograms shows the empirical comparison between JP-8 extracted from PBS and that from bovine plasma. Although the plasma is a much more complex medium than the PBS, the character of the extracted chromatograms is essentially identical; however, the extraction efficiency (as related to the internal instrument standard) is somewhat reduced. It ranges from 0.93% to 77%, depending on the compound recovered. By using the equations generated from the standard curves in Table 6, the concentration of each compound monitored in injection solution can be calculated. Furthermore, by following Eq. (2), with a known recovery efficiency for each compound monitored, the concentration of each compound in plasma can be calculated. Table 6 lists the concentrations of compounds of JP-8 extracted from bovine plasma (a

Table 5
Recovery efficiency of extraction–evaporation of JP-8 from pentane and DCM solution[a]

Compound	Recovery efficiency ±S.E.[a] (%)					
	C=0.16%		*C*=0.032%		*C*=0.016%	
	Pentane	DCM	Pentane	DCM	Pentane	DCM
Pentadecane	48.3±1.2	43.0±0.5	47.3±9.9	27.3±0.9	36.0±4.1	26.9±1.3
Tetradecane	46.7±1.1	44.2±0.8	44.1±8.4	26.6±1.0	34.9±2.8	32.3±4.6
Tridecane	47.6±1.8	46.0±1.2	37.3±5.6	26.1±1.1	30.3±2.5	28.6±1.9
Dodecane-d_{26} (ITSD)	100	100	100	100	100	100
Dodecane	46.2±3.1	47.9±1.2	30.8±4.7	26.6±1.7	22.7±0.9	26.7±0.4
Undecane	46.0±4.2	50.8±1.4	27.5±6.1	29.2±2.5	20.2±1.3	28.3±0.5
Decane	44.0±4.8	51.4±1.9	21.4±4.1	31.8±4.6	18.4±2.0	31.1±1.6
Nonane	38.5±3.5	43.6±1.0	12.9±2.2	23.2±3.6	14.8±2.2	26.0±1.4
Toluene	21.1±1.0	38.7±1.5	4.6±1.1	37.9±10.1	13.3±4.8	57.0±3.5
Benzene	18.2±5.7	27.0±0.5	8.5±0.0	22.0±0.8	21.5±10.1	24.8±2.1

[a] Abbreviations: DCM: dichloromethane, ITSD: internal standard, S.E.: standard error.



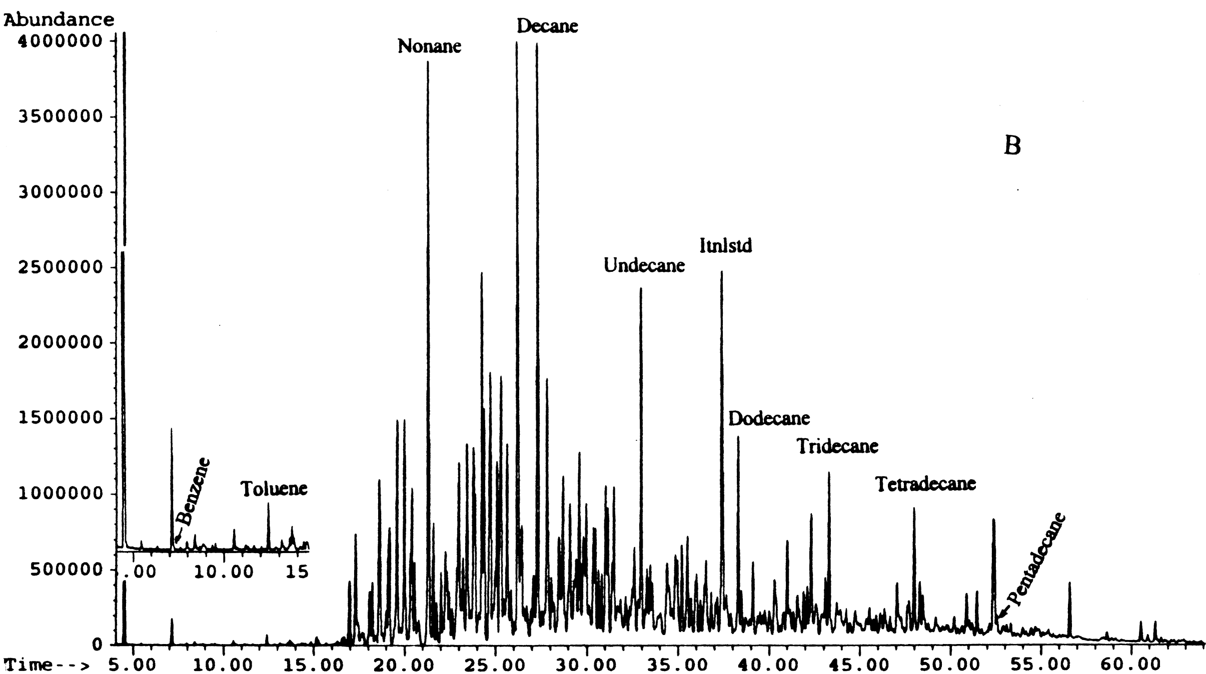


Fig. 3. GC–MS chromatograms of JP-8 extracted from (A) PBS and (B) bovine plasma.

Table 6
Equations of standard curves and calculation of concentration of extraction solutions

Compound	Equations from standard curves	Peak area ratio[a] (y)	Injection solution concentration (X)	Recovery (%) efficiency[a] (%)	Plasma concentration[b] (ng μl^{-1})
Pentadecane	$y=1.10\pm0.032\ X+0.067\pm0.15$	0.29	0.21	1.40	0.67
Tetradecane	$y=1.59\pm0.043\ X-0.42\pm0.20$	0.32	0.47	0.92	1.7
Tridecane	$y=1.82\pm0.064\ X-0.40\pm0.30$	0.58	0.54	1.07	1.03
Dodecane	$y=2.09\pm0.10\ X+1.05\pm0.46$	1.47	0.20	1.92	0.34
Undecane	$y=2.45\pm0.069\ X-0.32\pm0.32$	6.19	2.66	4.18	2.05
Decane	$y=2.74\pm0.088\ X-0.36\pm0.41$	22.92	8.50	8.39	3.27
Nonane	$y=2.46\pm0.072\ X-0.026\pm0.34$	34.33	13.97	12.07	3.73
Toluene	$y=104.3\pm4.11\ X-27.94\pm19.21$	12.58	0.39	22.79	0.06
Benzene	$y=83.73\pm2.97\ X-20.66\pm13.89$	3.62	0.29	69.89	0.013

[a] Data obtained from extraction of JP-8 from bovine plasma (0.016%) with pentane (18 ml). The control solution was in the same solution before extraction.
[b] Calculated according to Eq. (2).

0.016% JP-8 solution). Similarly, the concentrations of the compounds of JP-8 in blood samples from exposed human objects can be estimated by using the technique and methodology established above. The reduction in recovery efficiency from bovine plasma was believed mainly from the formation of emulsion during the extraction of plasma with pentane. We are currently exploring methods of improving the efficiency.

$$[VOC]=\frac{(X/RE)\cdot V_{\text{inj}}}{V_{\text{plasma}}} \quad (2)$$

where X = concentration of injection solution; RE = recovery efficiency; V_{inj} = volume of injection solution; V_{plasma} = volume of plasma solution.

3.7. Expected sensitivity in blood and the relationship to human exposure levels

According to the results of the standard curves for the various analytes, we can expect a typical sensitivity of about 0.4 ng μl^{-1} per compound in the final injection solution when using total ion acquisition (or full-scan) mass spectrometry. Assuming that initially a 6-ml sample of blood was extracted, that the final volume of solvent was 200 μl, and that the extraction efficiency was about 50%, then we can calculate an expected original blood concentration of about 0.03 ng/μl as the sensitivity of the basic method per compound. From prior research [17], we know that post-work breath concentrations of JP-8-related workers have means in the range of 10 to 85 ppbv for the C_9–C_{12} n-alkanes and 1 to 6 ppbv for benzene (depending heavily upon smoking status). Very few data are available for JP-8 or its constituents with respect to blood/air partition; however, we can estimate the sensitivity from published JP-10 data. (JP-10 is a single-component ram jet fuel with m.w. 136 and vapor pressure 1.18 kPa, which we assume to be similar to the midrange JP-8 compounds.) Given that the blood/air partition coefficient for JP-10 jet fuel is 52.5, and for benzene is 8.2 [33], then we can calculate that the breath concentrations corresponding to the blood measurement sensitivity limit of 0.03 ng μl^{-1} are 1 and 11 ppbv, respectively.

In the event that sensitivity becomes a major issue, as it might for incidentally exposed or unexposed control subjects, then sample extracts can be reanalyzed by using SIM acquisition for the MS detector. In Fig. 4 we show the contrast between a full-scan acquisition (Fig. 4A) and SIM acquisition (Fig. 4B) for a subset of the respective plasma extract chromatograms, each using 57 amu as the display single ion and a small range of the chromatogram around the tetradecane peak as the example. Note that we can expect about a 20-fold increase in quantitative sensitivity using SIM, primarily due to a great reduction in background noise, but that we

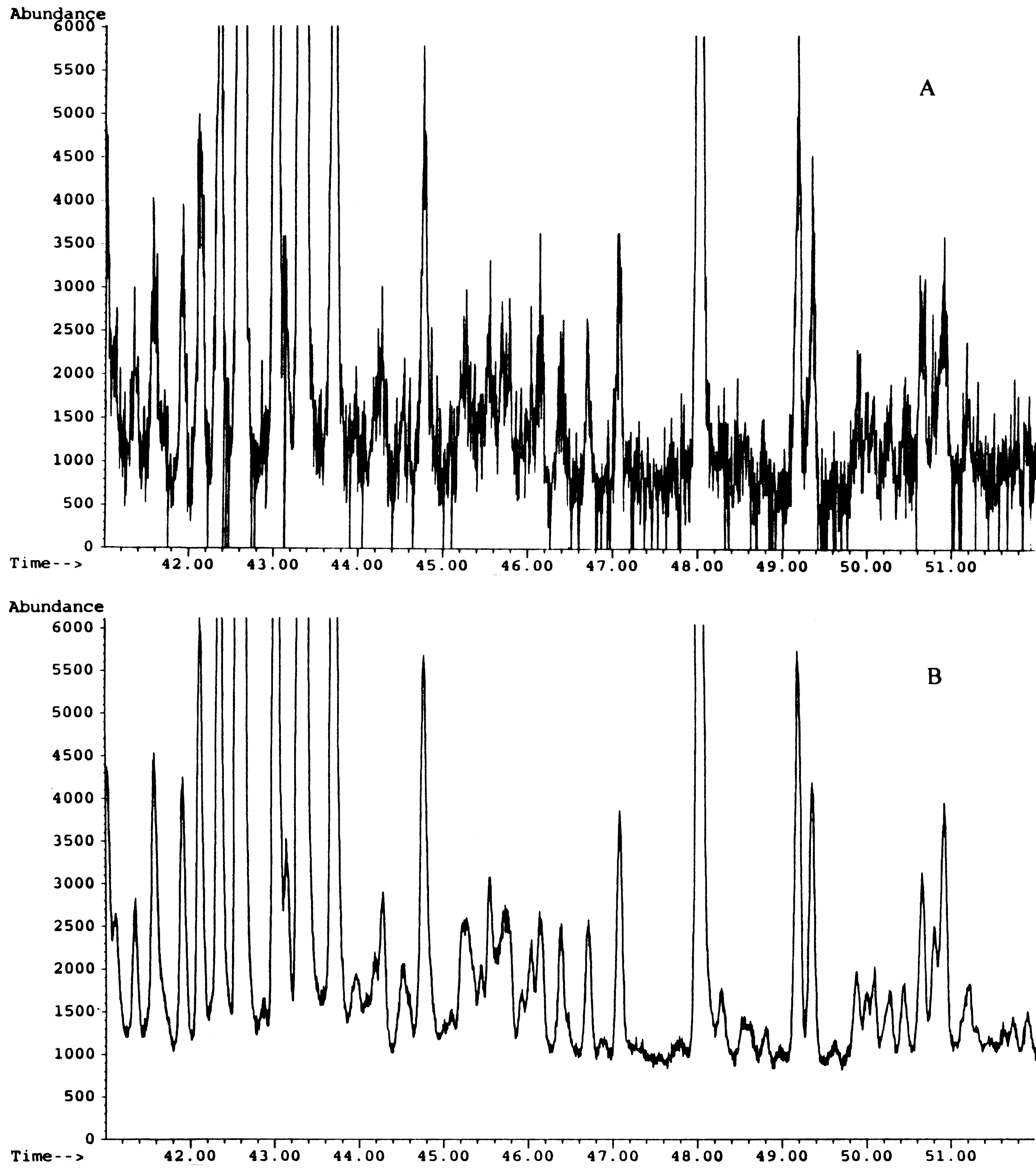


Fig. 4. Partial range of chromatograms of plasma extracts (from 41 min to 52 min, around the tetradecane peak, 48.1 min, as an example): (A) full-scan acquisition, (B) SIM acquisition, each using 57 amu.

consequently lose the capability to find and identify all the compounds that were not part of the pre-selected acquisition criteria. Therefore, we can estimate that our sensitivity for blood-borne analytes is about a 1.5-pg/μl equivalent blood concentration, which translates into breath concentration sensitivity estimates for typical background levels around 0.6 ppbv for the JP-8 alkanes.

4. Conclusions

A baseline method for liquid–liquid extraction of JP-8 from blood surrogate PBS solution has been successfully developed to accommodate a wide range of constituent compounds starting with the very volatile aromatic compounds (benzene and toluene) up to the semi-volatile compound pentadecane. For this purpose, we needed to make certain compromises involving temperature and injection technique. The procedure was assessed for recovery efficiency of representative VOCs in JP-8 jet fuel for two candidate extraction solvents, pentane and DCM. Each solvent presents particular technical issues to be considered. With pentane as the solvent and compounds of similar volatility as the analytes, we found that the injection temperature must be at or lower than the boiling point of pentane and that the injection needle must be immediately withdrawn after injection. With DCM as solvent, recovery efficiencies were not as susceptible to small changes in injection conditions; however, we encountered some interfering contamination, even in the highest purity grades, upon volume reduction; some solvent purification of DCM before use is needed to achieve sensitivity similar to that achieved with pentane use. The relatively low recovery of benzene may require the addition of a complementary technique such as SPME.

Overall, we have achieved a simple extraction and analytical method for assessing JP-8 content in a biological fluid. We anticipate that the application of this methodology to real blood samples will demonstrate unambiguous measures of blood-borne exposure from common exposure scenarios and that a defensible quantitative link can be forged between blood levels and exhaled breath concentrations. We found that the basic technique is sensitive enough to assess typical occupational exposures to JP-8, and that with a simple switch to SIM acquisition we can achieve increased sensitivity to measure background and incidental exposures. The eventual goal of this work is to extend the surrogate blood method to actual blood samples. Once the definitive link between quantitative blood and breath measurements is made, a non-invasive breath sample can eventually act as a quantitative substitute for an invasive blood measurement. In this way, we could extend biological monitoring for exposure to volatile organic compounds to a larger subject population than would be feasible for blood sampling alone. Certainly, blood would continue to be monitored on a subset of the subjects for quality assurance purposes.

Future work will include a refinement of the analytical methodology to improve sensitivity with full-scan MS. We will focus primarily on the injection technique, the maximum allowable injection volume, and chromatographic separation. Additionally, we will perform a rigorous evaluation of extraction from the real human blood matrix and define a specific field procedure and sample kit for collecting and extracting blood samples from human subjects.

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