Talanta, Vol. 38, No. 4, pp. 365–374, 1991
Printed in Great Britain. All rights reserved

0039-9140/91 $3.00 + 0.00
Copyright © 1991 Pergamon Press plc

QUANTIFICATION OF VOLATILE SOLVENTS IN BLOOD BY STATIC HEADSPACE ANALYSIS

RUSSELL L. DILLS,* STUART D. KENT, HARVEY CHECKOWAY and DAVID A. KALMAN
Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195, U.S.A.

(*Received* 8 *August* 1990. *Revised* 19 *September* 1990. *Accepted* 5 *October* 1990)

Summary—A static headspace method for determination of volatile solvents in blood was developed. The solvents determined were 1,1,1-trichloroethane, toluene, xylene (*o*-, *m*- and *p*-), ethylbenzene, styrene, α-methylstyrene and 4-methylstyrene at concentrations ranging from 0.01 to 1 μg/ml. Internal standard calibration was used. Parameters affecting sensitivity and precision were determined and optimized.

Volatile organic solvents are common contaminants in the environment and work place. Recent human exposure to these contaminants is most accurately assessed by monitoring the concentration of individual solvents in the blood. One method of quantifying solvent concentration in blood is headspace analysis, in which analytes dissolved in blood are partitioned between the blood and air in a closed system and the concentrations of analytes in the airspace (headspace) over the blood are determined. The concentrations of analytes in the headspace are proportional to their concentrations in the blood.[1]

As part of our research in monitoring exposures to environmental and work place contaminants, we wanted a general screening method for blood that could detect low levels of common solvents. The desired quantification limit was approximately the level expected in an average human exposed to a concentration equal to 1% of the occupational guideline suggested by the American Conference of Governmental Industrial Hygienists. Ramsey *et al.*[2,3] have developed a method for screening volatile organic compounds in blood by headspace analysis. Their method was created for diagnostic use in high-exposure situations occurring with solvent abuse and acute poisoning. They used low-resolution (packed-column) gas chromatography (GC) and did not automate the method. We have therefore developed and validated an automated method for headspace analysis of blood from humans with low-level exposure to solvents.

The method described in this report differs from previously published analyses in that: (*a*) the quantification limit is lower (as low as 0.01 μg/ml in blood); (*b*) the system is fully automated, permitting up to 20 hr of unattended operation; (*c*) the range of volatility for analytes is wide (b.p. 12–170°); and (*d*) high-resolution gas chromatography is used, permitting discrimination between xenobiotics and background agents.

EXPERIMENTAL

Materials

"Nanograde" toluene was obtained from Mallinckrodt (St Louis, MO). 1,1-Difluoro-1,2,2-trichloroethane was purchased from PCR (Gainesville, FL). 1,1,1-Trichloroethane (TCE), ethylbenzene, *p*-xylene (gold label; inhibited with 10–15 ppm 4-*tert*-butylcatechol), *m*-xylene, *o*-xylene (HPLC grade), styrene (gold label; inhibited with 10–15 ppm 4-*tert*-butylcatechol), 4-chlorofluorobenzene, α-methylstyrene (inhibited with 15 ppm 4-*tert*-butylcatechol), and 4-methylstyrene (inhibited with 0.1% by weight hydroquinone) were bought from Aldrich Chemical Company (Milwaukee, WI). Dimethylsulfoxide (DMSO) was obtained from Burdick and Jackson (Muskegon, MI). Analytical-reagent grade chemicals were used unless otherwise indicated. The styrenes and 1,1-difluoro-1,2,2-trichloroethane were kept refrigerated because of their tendency for slow degradation. The chemicals used for standards were analyzed by gas chromatography/mass spectrometry (GC/MS) for impurities. We noticed some variation of purity in the chemicals,

*Author for correspondence.

depending on source and lot. The sources and grades indicated above were satisfactory.

Gas chromatography

A capillary gas chromatograph with a flame ionization detector (Hewlett–Packard model 5880A; Avondale, PA) was used. The nitrogen make-up gas flow to the detector was approximately 30 ml/min. The detector temperature was 250°. Liquid carbon dioxide was the coolant for sub-ambient temperature programming and was controlled by the sub-ambient temperature control-option available for the 5880A gas chromatograph. The oven temperature control was turned off for the duration of the analyte transfer (5 min), by commands in the run table.

The multi-ramp oven temperature program was as follows: −10° for 5.2 min initial temperature; heating to 40° at 15°/min followed by an 8-min isothermal period; heating to 65° at 4°/min; ballistic heating to 220° followed by a 6-min isothermal period (for post-analysis column conditioning).

A DB-WAX capillary column (30 m × 0.32 mm i.d., 0.25 mm film thickness; J&W Scientific, Folsom, CA) was directly connected to the transfer line of the headspace sampler with a zero dead-volume union (ZU.5TJ with graphite ferrules; Valco Instruments Company, Inc., Houston, TX). If the union became cooled, during sub-ambient temperature programming, for example, split peaks were observed in the chromatogram. To prevent this, the union was placed inside the heated zone of the capillary injector, ensuring that the union remained at 150°. The linear velocity of the helium carrier gas was 20 cm/sec at 150°.

Headspace sampler

A Hewlett–Packard 19395A headspace sampler was used with an 18906A constant heating-time accessory. The headspace sampler hardware and operation have been described by Wylie,[4] as has the constant heating-time apparatus.[5] In brief, the headspace sampler maintained samples at a constant temperature for a preselected period prior to sampling. To sample the headspace, the sample vial was first pressurized with helium admitted through a needle piercing the septum of the vial. The vial was then vented, whereby a positive-pressure transfer of the helium/headspace mixture was made to a volumetric injection loop (1 ml). When the loop had been filled, it was switched into the carrier gas stream and its contents swept into the gas chromatographic column. Operational parameters for analyses with the headspace sampler are shown in Table 1.

Cryotrap

The cryotrap was a brass tube (13 × 0.635 cm o.d.) suspended in the gas chromatograph oven. The gas chromatographic column was centered in the brass tube with graphite ferrules swaged onto the tube ends. When there was a tight seal between the column and the ferrules, multiple split peaks were observed in the chromatogram. We attributed this phenomenon to the existence of cold spots where the column contacted the ferrules. To avoid this phenomenon, the holes drilled in the ferrules were oversized.

Liquid nitrogen was the coolant for the cryotrap and entered it counter-current to the gas chromatographic column carrier-gas flow. The liquid-nitrogen supply was controlled by a solenoid valve that was activated when the headspace sampler probe descended. The cryotrap was cooled for 7 min and then warmed by compressed air until the trap temperature reached approximately 40°. The compressed air was controlled by a solenoid valve activated through the run table.

The coolant flow was regulated to maintain a temperature of −120° (temperature controller, Model 310; Omega Engineering, Stamford, CT). The thermocouple (type J) was attached to a brass screw opposite the liquid-nitrogen inlet. A second thermocouple (type K) was used to monitor the outer surface temperature of the trap. This thermocouple was wired to the "auxiliary temperature 2" sensor input on the 5880A gas chromatograph.

Sample preparation

The recommendations of the Centers for Disease Control for the handling of blood were

Table 1. Operational parameters for headspace analyses

Parameter	Value
Sample heating time	74 min
Sample temperature	60°C
Sampling valve temperature	65°C
Analysis time	37 min
Vial overpressure	1.2 bar*
Carrier gas pressure	1.4 bar*
Sample loop size	1 ml
Vial pressurization duration	1 min
Vial venting duration	2 sec
Injection duration	5 min

*Above atmospheric pressure.

followed.[6] Blood samples were taken in "vacutainers" containing sodium citrate as an anticoagulant. Samples were kept at approximately 4° until sample preparation. The "vacutainers" were filled as completely as possible, to minimize the headspace above the blood sample.

To prepare a sample for analysis, 2 ml of water containing 2 mg of sodium azide were placed in a 10-ml crimp-top vial (23 mm o.d., 47 mm in height; Alltech Associates, Deerfield, IL) and frozen. When the ice in the vial began to thaw slightly, 1 ml of blood was pipetted onto the ice with an Eppendorf Combitips pipettor (Brinkman Instruments, Westbury, NY) and an 18-gauge needle. A Teflon-lined butyl rubber septum (Hewlett–Packard, part no. 9301-0976) and an aluminum seal (20-mm o.d. seal without centers; Alltech Associates) were immediately placed on the vial and the seal was crimped. We found that these septa are the most resistant to gas leakage. Once sealed, the vials were stored at 4° until analysis.

Immediately prior to analysis, 10 μl of an internal standard (a solution of 61 μg of 4-chlorofluorobenzene and 78 μg of 1,1-difluoro-1,2,2-trichloroethane per ml in DMSO) were added to the prepared blood sample. The internal standard solution was carefully injected under the surface of the blood–water mixture with a 500-μl syringe (gas-tight, Luer tip; Hamilton Company, Reno, NV), a PB-600 repeating dispenser (Hamilton), and a 26-gauge needle. The final concentrations of internal standards in the blood were 0.61 μg of 4-chlorofluorobenzene and 0.77 μg of 1,1-difluoro-1,2,2-trichloroethane per ml.

Standard preparation

For method validation experiments, standard curves and blanks, out-of-date human blood was used as the matrix. Out-of-date blood was obtained from a local blood bank and was one or two days past its expiry date. The blood was not hemolyzed or septic.

With each set of samples, a set of spiked blood standards was analyzed and a standard curve constructed. The blood was spiked by the same procedure as described for the addition of internal standard to samples. The concentrations of each analyte in the spiking solutions were approximately 1.0, 5.0, 10, 50 and 100 μg/ml which gave, respectively, concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 μg/ml in the blood. Along with the spiked blood standards, a blood sample containing only internal standard was analyzed as a blank. The result from this blank analysis was used as the zero point in the standard curve.

Standard curves

Standard curves were prepared daily. Peak-area ratios (of analyte to internal standard) were computed. Weighted least-squares linear regression of blood concentration of analyte *vs.* area ratios yielded the slopes and intercepts. When non-weighted linear regression was used, the highest concentration data points had undue influence, causing poor fits of the regression lines at the lower concentration data points. The square-root of the reciprocal of the concentration was used as the weighting factor. Heteroscedasticity was reduced by this weighting scheme. Regressions were performed with the MGLH module of Systat (Systat, Inc., Evanston, IL).

RESULTS AND DISCUSSION

GC parameters

The choice of satisfactory columns was limited. Our primary criterion for chromatographic separation was resolution of the xylene isomers and ethylbenzene. Only columns with a polyethylene glycol stationary phase achieved resolution of these solvents. Columns of 0.25 mm i.d. occasionally became blocked with ice, a problem that was avoided by using 0.32 mm i.d. columns (as had been reported by others[4,7]). A film thickness of 0.25 μm was chosen to maximize resolution between the xylenes. Though a DB-wax column afforded resolution of the solvents tested, it did not resolve the most volatile components (Fig. 1).

The connection of the transfer line from the headspace sampler to the gas chromatographic column had considerable influence on the sensitivity and precision of the headspace analysis. When the transfer line was inserted into the split/splitless injector port through the septum, and the injector was operated in the split mode as recommended in the headspace sampler manual,[8] the sensitivity was not sufficient for our needs. As the split ratio was decreased, the sensitivity increased. However, the precision decreased, and at a split ratio of 10 the coefficient of variation was approximately 20–30%. When the injector was used in the splitless mode, no increase in sensitivity was

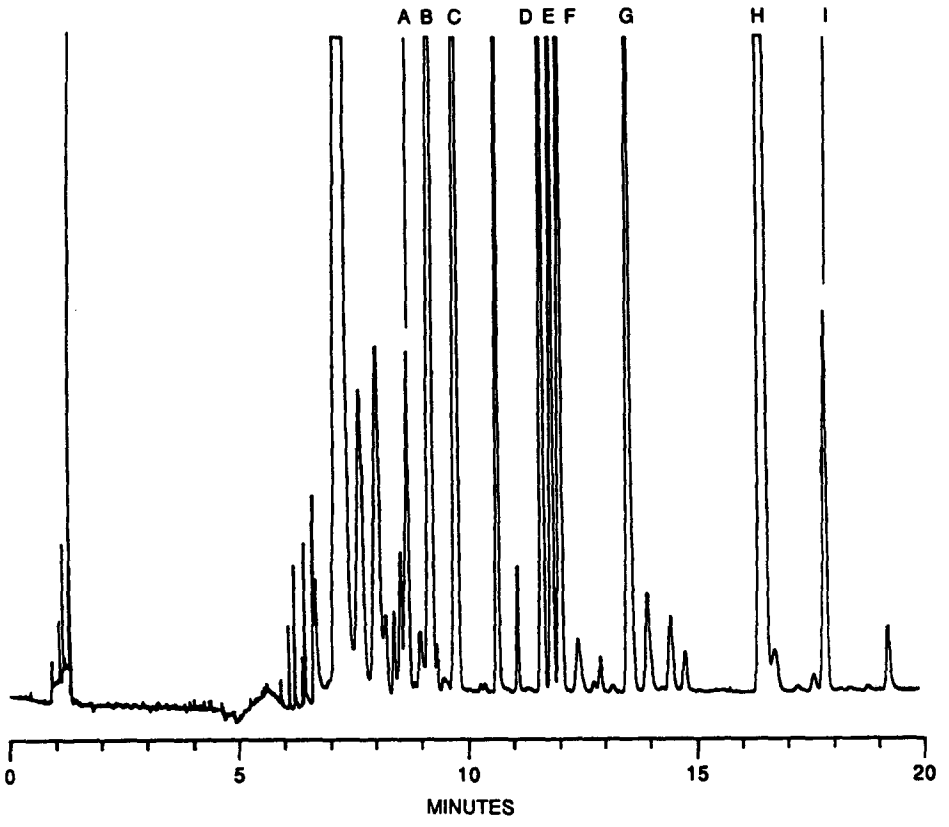

Fig. 1. Chromatogram of a spiked blood sample. The concentration of the analytes was approximately 0.1 μg/ml. Transfer of analytes to the gas chromatographic column was between 0 and 5.7 min. A = 1,1,1-trichloroethane; B = 1,1-difluoro-1,2,2-trichloroethane; C = toluene; D = ethylbenzene; E = *p*-xylene; F = *m*-xylene; G = *o*-xylene; H = 4-chlorofluorobenzene; I = styrene. The methylstyrenes were eluted between 20 and 25 min. 1,1-Difluoro-1,2,2-trichloroethane and 4-chlorofluorobenzene were used as internal standards.

gained, because slow transfer of the analytes from the large volume injector into the capillary column caused gross tailing and distortion of the chromatographic peaks. The solution to the sensitivity and precision problem was to connect the column and transfer line directly. This increased the sensitivity while maintaining the precision at acceptable levels (coefficient of variation < 10%).

Because the gas chromatograph inlet was bypassed by direct connection of the gas chromatographic column to the transfer line, the headspace sampler supplied the gas chromatograph with carrier gas. Assuming continuous and instantaneous mixing of the contents of the 1 ml sample loop with carrier gas incoming at a flow-rate of 1.0 ml/min, the time required for transfer of 99% of the analytes to the column (4.6 min) was calculated by use of a gas dilution equation.[9] This calculation neglected the small dead volume of the transfer line (0.13 ml). Therefore, the transfer time (injection time) was set at 5 min.

Cryotrap

Because of the 5 min transfer of the headspace mixture, a cryotrap was necessary to focus the analytes that were more volatile than *m*-xylene into a narrow region of the gas chromatographic column. Without the cryotrap, *p*-xylene, *m*-xylene and ethylbenzene were poorly resolved, toluene was not resolved from endogenous volatile compounds in blood, and TCE was not detectable.

The optimum arrangement of coolant flow and heating-air flow was determined. Peak width was minimized when the coolant flow was counter-current to the carrier-gas flow and heating-air flow. Kolb *et al.*[7] noted that this configuration formed a temperature gradient in the column and reduced the peak width.

Occasionally analytes escaped collection in the cryotrap. This was typically caused by coolant supply problems and was evidenced by breakthrough of the untrapped compounds during the sample transfer. Compounds re-

tained on the column at room temperature, such as styrene, were unaffected by trap failure.

Headspace sampler parameters

The optimum conditions for headspace sampling were determined in a series of experiments with either blood or water as the sample matrix. Blood was used as the matrix when the matrix was likely to influence the optimization. Both response and precision were evaluated during the optimization. The parameters optimized were heating time, sample temperature, the method used to fill the sample loop, equilibration time for headspace vial pressurization, the overpressure and pressurization time.

The sensitivity of headspace analyses has been improved by heating the sample matrix, which increases the concentration of analytes in the gas phase.[1] To determine the effect of sample temperature on the sensitivity and precision, vials containing either 3 ml of water or 1 ml of blood and 2 ml of water were spiked and the headspace concentrations of the analytes were determined. Sample temperature had different effects upon the response for analytes in blood or water as matrix (Fig. 2). For water, the response increased with temperature over the range 40–90° while for blood, the response was maximal at 60°. At higher temperatures, blood coagulated prior to sampling. The partitioning of analyte from a solid (coagulated blood) to air was less efficient than from a liquid to air. The precision, in all cases, was inversely proportional to the response.

To evaluate the effect of sample heating time on the response and precision, replicate headspace vials containing toluene, the xylenes, internal standard and styrene in a water matrix

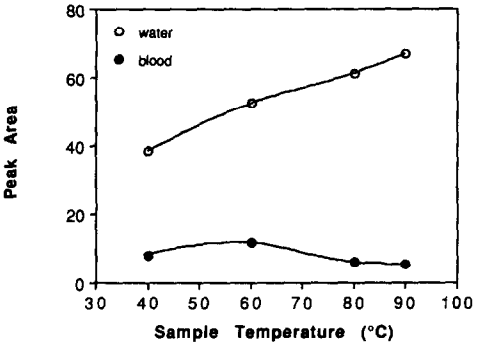

Fig. 2. The effect of sample temperature on the response for styrene. Headspace samples were equilibrated for 80 min prior to analysis. Two different sample matrices, water and a 1:2 mixture of blood and water, were examined in this experiment. Similar results for other analytes were observed in these two matrices.

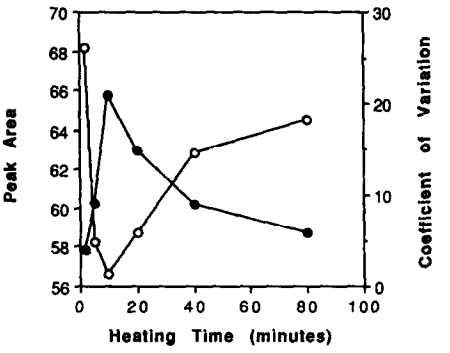

Fig. 3. The effect of heating time on response and precision for styrene. Headspace samples were prepared at room temperature and then heated. The heating time is the length of time the sample remained at 60° prior to analysis. Thermal equilibrium was achieved in approximately 5 min. Three replicate analyses were performed for each heating time. Other analytes exhibited similar trends in response and precision. (●) Coefficient of variation; (○) peak area.

were prepared and analyzed with the headspace sampler. As the headspace vials warmed to 60°, a new equilibrium concentration of analyte vapor in the headspace was approached. The vials reached thermal equilibrium in approximately 5 min. However, the concentration of analyte vapor in the headspace continued to rise for at least 80 min (Fig. 3). The coefficient of variation was inversely proportional to the concentration of analyte vapor (Fig. 3). Experiments with blood as matrix showed similar behavior. Thus, the optimum heating time for a sample would be at least 80 min.

Because of the mechanics of the constant heating-time apparatus, the heating time was required to be some multiple of the run time. Each sample was heated for two runs (74 min). Although a longer heating time would have allowed the analytes to reach equilibrium, the next allowable duration (111 min) would have significantly decreased the number of samples analyzed per day. Even though the analytes in the headspace vial were not in complete thermodynamic equilibrium between the phases, reproducible measurements could be made with the constant heating-time apparatus, because the heating period before sampling of the headspace was the same for each vial.[5]

A hyperbolic relationship between response and overpressure was derived from the basic gas laws. Response was proportional to the concentration in the headspace sample (C_s), in this case,

$$C_s = C_i\left(\frac{P_i}{P_i + P_a}\right) \qquad (1)$$

where C_i is the concentration of analyte in the headspace prior to pressurization, P_i the initial headspace pressure (atmospheric), and P_a the overpressure (pressurization above atmospheric). This relationship was confirmed experimentally (data not shown). In order to deliver at least 1 ml of helium/headspace mixture to the sample loop, P_a had to be greater than approximately 0.2 bar. There was no discernible relationship between the precision and overpressure.

We confirmed that more analyte was contained in the sample loop when the loop and vial were not completely depressurized as indicated in the instrument manual.[8] Therefore, the duration of sample venting was set to 2 sec, an insufficient time for complete depressurization. However, we noted that better reproducibility was achieved if P_a was 0.8–1.2 bar, which was higher than the overpressure optimum for sensitivity.

The optimum duration for pressurization and the time allowed for pressure equilibration in the sample vial were determined by replicate analysis of water spiked with analytes. Pressurization required at least 30 sec; shorter times resulted in poor precision and reduced sensitivity. Equilibration time did not affect the sensitivity. However, precision was highest with equilibration times greater than 30 sec.

Sample preparation

For valid measurements, the septum sealing the headspace vial must not allow analyte to escape. By pressurizing the vial and then submerging it under water, we evaluated the integrity of the septum seal, for silicone rubber–Teflon and butyl rubber–Teflon composite septa. Silicone rubber septa leaked after a single puncture with the 26-gauge needle used to add internal standard to the sample. Furthermore, this type of septum did not always form an air-tight seal. However, the butyl rubber–Teflon septa performed excellently and did not leak under 3 bar pressure even after the vial had been punctured five times. Gill *et al.*[3] studied the loss of analytes from headspace vials and concluded that either Teflon- or aluminum-lined septa offered the best stability.

The effect of sample volume on response was determined experimentally. Blood (10 ml) was spiked with toluene, ethylbenzene, the xylenes, styrene, α-methylstyrene, and 4-methylstyrene, and a series of headspace vials was prepared, with different volumes of spiked blood. The samples were then analyzed according to the standard procedure.

The response changed with blood volume as expected, but the magnitude of the change and the optimum volume were dependent on the analyte (Fig. 4). Apparently the compounds that are partitioned to the greatest extent into the air were most affected by the blood volume. Small variations in the blood volume dispensed would be more likely to affect the precision for compounds with a lower affinity for blood.

The relationship between blood volume and response was derived from the mass balance equation describing the partitioning of analyte between the two phases in the headspace vial. The mass balance equation is:

$$V_b C_i = V_b C_b + V_a C_a \qquad (2)$$

where V_b is the volume of blood and water mixture in the headspace vial, C_i the concentration of analyte in the blood before partitioning, C_b the concentration of analyte in the blood after partitioning, V_a the volume of air (gas phase) in the headspace vial and C_a the concentration of analyte in the gas phase of the headspace vial after partitioning. By definition the partition coefficient between the blood and water mixture and air ($k_{b/a}$) is given by $k_{b/a} = C_b/C_a$, and the total vial volume is $V_t = V_b + V_a$; substituting these variables in equation (2) and solving for C_a gives:

$$C_a = \frac{V_b C_i}{V_b(k_{b/a} - 1) + V_t} \qquad (3)$$

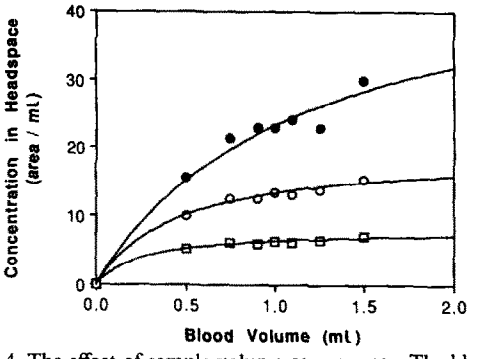

Fig. 4. The effect of sample volume on response. The blood was spiked with a solvent mixture (0.1 μg/ml) and various volumes were analyzed according to the procedure described. The standard volume of blood analyzed was 1 ml, to which 2 ml of water were added. The relationship between the blood volume and response is given by equation (3), but as $k_{b/a}$ was not known, least-squares non-linear regression was used to fit an equation of the form $C_a = V_b/(aV_b + b)$, where a and b are constants, to the results. The lines are the regression lines. Similar relationships held for all compounds analyzed. (●) Toluene; (○) styrene; (□) 4-methylstyrene.

The liquid phase is the blood–water mixture.

The sensitivity in headspace analyses can be increased by adding compounds that increase partitioning of the analyte into air. Inorganic salts have greatly increased the sensitivity of analyses for polar chemicals, such as alcohols[1,10-12] and water-miscible ketones and aldehydes.[1] However, there is very little effect, if any, with non-polar chemicals.[1,13,14] We have examined several additives which alter the physicochemical structure of blood. In these experiments, 1 ml of blood was placed in a headspace vial with 2 ml of an additive. Additives were 0.9% saline water, 8*M* urea, 1*M* aluminum sulfate and 1 ml of water followed by 1 ml of 4.0*M* ammonium sulfate. Unadulterated blood (1 ml) was analyzed as a control. Three replicates were analyzed for each additive and control.

The additives which caused the blood to coagulate, ammonium sulfate and aluminum sulfate, increased the variability (Table 2). The importance of preventing blood from coagulating was noted previously, but without explanation.[15] The additives which lysed the blood cells, *i.e.*, water and 8*M* urea, decreased the variability, as did saline which, in essence, thinned the blood. The response was increased most by addition of water to the blood. The concentrations of chloroform, toluene and styrene in the headspace were previously shown to be increased by hemolysis of blood by water.[16] Hemolysis probably increased the response because it retarded coagulation of the blood. Solid–gas phase partitioning of analytes is less efficient than liquid–gas phase partitioning. In addition, hemolysis probably increased the availability of analytes from the blood cells, which apparently contain higher concentrations of solvents than the surrounding plasma does.[17,18]

The effect of sample storage upon response was evaluated. Replicate samples of spiked blood were prepared according to the standard procedure and stored at 4°. Sets of samples were analyzed on the day of preparation, and then 24 and 72 hr later. The response did not decrease during 72 hr of storage. Gill *et al.*[3] showed that samples stored without headspace in bottles with Teflon-lined caps did not lose analyte during storage for 30 days.

Standard preparation

Four methods have previously been used to spike blood: direct addition of neat analyte to blood, addition of an aqueous solution of analyte to blood, addition of a blood solution of analyte to blood and addition of analyte in a water-miscible vehicle to blood. The primary goals in preparing standards for headspace analysis were quantitative transfer of the analyte into the liquid phase and rapid achievement of physicochemical equilibrium. Not all these methods could achieve these objectives.

Equilibrium was reached slowly (>24 hr) when immiscible liquids were mixed to prepare standards of halogenated hydrocarbons[19,20] and aromatic hydrocarbons[20] in water. Furthermore, Radzikowska-Kintzi and Jakubowski[15] indicated that introduction of μl volumes of undissolved hydrocarbons into blood samples caused problems with precise dosage. Formation of a micro-emulsion of immiscible solute dispersed in aqueous solution was discussed by Sanemasa *et al.*[21] Solutions of analytes in blood have been used to spike samples.[3,15] This ap-

Table 2. The effect of additives to the headspace blood sample on response and precision

	Additive											
	None		Saline		Water		$(NH_4)_2SO_4$		$Al_2(SO_4)_3$		Urea	
Compound	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %
Toluene	113	22	129	4	146	6	72	16	128	21	119	4
Ethylbenzene	89	17	102	3	116	7	62	17	113	28	103	5
p-Xylene	88	16	105	2	113	7	61	19	111	31	97	5
m-Xylene	88	16	104	3	115	7	62	20	108	29	96	5
o-Xylene	84	14	100	2	107	6	62	23	115	29	83	4
4-Chloro-fluorobenzene	75	13	83	4	97	6	50	20	79	26	71	3
Styrene	66	9	68	3	76	5	47	28	32	80	41	2
α-Methylstyrene	51	7	61	2	70	6	47	32	29	87	38	3
4-Methylstyrene	33	10	49	5	42	5	33	34	23	86	26	3

Response was the mean peak area from three replicate analyses. Two ml of additive were added to 1 ml of blood prior to the analyses, except for $(NH_4)_2SO_4$ [where 1 ml of water was added, followed by 1 ml of $(NH_4)_2SO_4$ solution]. Aqueous solutions were: 0.9% saline, 4.0*M* $(NH_4)_2SO_4$, 1.0*M* $Al_2(SO_4)_3$ and 8*M* urea. CV is the coefficient of variation.

proach appeared to give good results, with a reported stability of 3 weeks for solutions of aromatic hydrocarbons and TCE. However, storage of blood solutions is problematic for some solvents, because of blood-induced degradation, for example, of esters[22] and ethanol.[11] Furthermore, blood deteriorates during storage and this is accelerated by sepsis.

We prefer to use a spiking solution made with a water-miscible vehicle. Addition of a second solute to water, in this case the vehicle, has been shown to change the solute–vapor equilibrium constant.[21,23] These secondary solutes have an influence only when their mole fraction is greater than 5×10^{-3}.[23] For this reason, we used 10 μl of vehicle, which corresponded to a DMSO mole fraction of 1.5×10^{-3} in the aqueous phase of the headspace vial.

We initially used methanol as the vehicle but found it unsatisfactory because it was eluted early in the chromatogram and interfered with the quantification of TCE and toluene. Therefore, we evaluated several water-miscible solvents as substitutes, namely formamide, DMSO, dimethylformamide, 3,3′-oxydipropionitrile, hexamethylphosphoramide and propylene glycol. Formamide and dimethylformamide contained impurities and the solutions were not stable. A major peak, possibly for a contaminant in the solvent, appeared early in the chromatogram when 3,3′-oxydipropionitrile was used. Their high viscosity made it difficult to transfer solutions in propylene glycol. Hexamethylphosphoramide caused baseline disturbances on the chromatogram and posed additional handling problems because of its toxicity.

DMSO proved satisfactory as the vehicle. It was eluted very late in the chromatogram and did not interfere with any of the analytes. Solutions of the analytes in DMSO were stable when stored frozen at 4°. Freezing and thawing of DMSO solutions of analytes did not affect the analyte concentration. The DMSO solutions used contained only minor (<0.1%) impurities or decomposition products which were eluted before TCE. The impurities were identified by GC/MS as dimethylsulfide and methylsulfide.

Because the internal standard was used to correct for recoveries of analytes, it had to behave similarly to the analytes. We found that one potential internal standard, 4-chlorofluorobenzene, behaved similarly to all the aromatic analytes in method development experiments, but TCE and 4-chlorofluorobenzene exhibited different recovery and response as the experimental parameters were changed. To find an internal standard for TCE we therefore examined compounds structurally related to it. Few compounds satisfied the constraint of being chromatographically resolvable from the analytes and common blood components and not being present as an environmental or work place contaminant. 1,1-Difluoro-1,2,2-trichloroethane satisfied these constraints but was not ideal, owing to its slow degradation in aqueous solution at elevated temperatures (60°).

Analytical performance

Standard curves were obtained for six levels of analyte in spiked samples of human blood: 1, 0.5, 0.1, 0.05, 0.01 and 0 μg/ml. Over this range the standard curve was linear (Table 3). TCE was not reliably quantified below 0.1 μg/ml because of interference from the peaks of endogenous blood substances. Styrene and toluene had measurable background concentrations; these concentrations fluctuated, depending on the batch of blood used for standard preparation, but were less than 0.1 μg/ml. The precision of response was calculated for each calibration point (Table 4). The coefficient of variation was generally less than 5%.

CONCLUSIONS

Static headspace analysis is an accurate and useful method for quantifying solvents in blood. Studies performed during development of the method showed that the headspace methodology must be optimized specifically for blood, rather than by use of parameters derived from headspace experiments with aqueous media. For example, procedures used to increase sensitivity in water analyses, such as salting-out, decreased the sensitivity for blood analyses because of their coagulative effects on blood.

Table 3. Linearity of the calibration curves [peak-area ratio, analyte to internal standard, plotted *vs.* concentration (μg/ml)]

Compound	Intercept*	Slope*	r^2
TCE	0.014 ± 0.004	0.41 ± 0.01	0.981
Toluene	−0.034 ± 0.001	0.36 ± 0.005	0.998
Ethylbenzene	0.000 ± 0.002	0.45 ± 0.01	0.993
p-Xylene	−0.002 ± 0.002	0.45 ± 0.01	0.996
m-Xylene	−0.011 ± 0.002	0.48 ± 0.01	0.996
o-Xylene	−0.006 ± 0.002	0.44 ± 0.01	0.996
Styrene	−0.062 ± 0.003	0.85 ± 0.01	0.995

*Estimated value ± standard error. Weighted least-squares linear repression (weight = $1/\sqrt{\text{concentration}}$).

Table 4. Precision of the method estimated from three replicates measurements of peak-area ratio for analyte/internal standard (ND = not detected)

Concentration in blood, μg/ml	TCE		Toluene		Ethylbenzene		*p*-Xylene		*m*-Xylene		*o*-Xylene		Styrene	
	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %
1.00	2.49	3.0	2.86	1.5	2.22	1.2	2.24	1.5	2.11	1.8	2.29	1.64	1.28	0.8
0.50	1.07	3.1	1.45	3.5	1.14	5.2	1.11	1.3	1.05	1.2	1.12	4.87	0.64	1.3
0.10	0.19	6.9	0.40	4.6	0.18	11.6	0.19	3.4	0.22	4.5	0.23	1.20	0.18	2.9
0.05	ND		0.25	2.9	0.07	3.8	0.09	4.3	0.11	1.1	0.10	16.5	0.12	3.2
0.01	ND		0.12	7.0	0.03	1.9	0.03	2.6	0.05	3.6	0.04	5.79	0.09	3.6
0.00	ND		0.09	3.2	ND		ND		ND		ND		0.08	3.2

For optimum sensitivity and precision, standards and samples must be prepared and manipulated in a way that minimizes loss of the volatile analytes. For valid quantification with the internal standard method of calibration, the physicochemical properties of the internal standard must be closely matched to those of the analytes; an internal standard that would be adequate for all the compounds investigated could not be found.

Acknowledgements—This research was supported by grants from the National Institute of Environmental Health Sciences (NIEHS ES 04696) and the National Institute of Occupational Safety and Health (NIOSH OH-02629). Support was also provided by the Workmen's Compensation Fund of the State of Washington, which is administered by the Washington Department of Labor and Industries. We thank P. L. Wylie (Hewlett–Packard) for his helpful suggestion on interfacing the headspace sampler to the gas chromatographic column.

REFERENCES

1. B. V. Ioffe and A. G. Vitenberg, *Headspace Analysis and Related Methods in Gas Chromatography*, Wiley–Interscience, New York, 1982.
2. J. D. Ramsey and R. J. Flanagan, *J. Chromatog.*, 1982, **240,** 423.
3. R. Gill, S. E. Hatchett, M. D. Osselton, H. K. Wilson and J. D. Ramsey, *J. Anal. Toxicol.*, 1988, **12,** 141.
4. P. L. Wylie, *Chromatographia*, 1986, **21,** 251.
5. *Idem, Headspace Analysis with Constant Sample Heating Time, Application Note 228-58*, Hewlett–Packard, Avondale, PA, 1988.
6. *Centers for Disease Control, MMWR*, 1987, **36,** Sup. 2S, 1.
7. B. Kolb, B. Liebhardt and L. S. Ettre, *Chromatographia*, 1986, **21,** 305.
8. *Operating and Service Manual for the HP 19395A Headspace Sampler*, Hewlett–Packard, Avondale, PA, 1988.
9. G. O. Nelson, *Controlled Test Atmospheres*, p. 100. Ann Arbor Science, Ann Arbor, MI, 1971.
10. M. Termonia, A. De Meyer, M. Wybauw and H. Jacobs, *J. HRC. & CC*, 1982, **5,** 377.
11. D. S. Christmore, R. C. Kelly and L. A. Doshier, *J. Forensic Sci.*, 1984, **29,** 1038.
12. M. Pelletier, M. Martin and W. Haerdi, *Mitt. Geb. Lebensmittelunters. Hyg.*, 1983, **74,** 339.
13. H. Kersting and H. Heberer, *Z. Gesamte Hyg. Ihre Grenzgeb.*, 1986, **32,** 552.
14. P. L. Wylie, *Trace Analysis of Volatile Compounds in Water, Using the HP 19395A Headspace Sampler, Application Note 228-40*, Hewlett–Packard, Avondale, PA, 1985.
15. H. Radzikowska-Kintzi and M. Jakubowski, *Int. Arch. Occup. Environ. Health*, 1981, **49,** 115.
16. M. Jakubowski, H. Radzikowska-Kintzi and G. Meszka, *Bromatol. Chem. Toksykol.*, 1980, **13,** 263.
17. D. D. Davies, R. B. Douglas and S. Ling, *Br. J. Anaesth.*, 1986, **58,** 1440.
18. M. J. Kamlet, R. M. Doherty, J. M. Abboud, M. H. Abraham and R. W. Taft, *Chemtech*, 1986, **16,** 566.

19. L. K. Jansen, *Determination and Use of Solute/Vapor Equilibrium Constants for Chlorinated Hydrocarbons, with Application to Industrial Hygiene Practice, Master's Thesis*, University of Washington, 1987.
20. M. E. McNally and R. L. Grob, *J. Chromatog.*, 1984, **284**, 105.
21. I. Sanemasa, Y. Miyazaki, S. Arakawa, M. Kumamaru and T. Deguchi, *Bull. Chem. Soc. Japan*, 1987, **60**, 517.
22. P. Pfäffli and N. Suartling, *J. Chromatog.*, 1985, **345**, 386.
23. C. Munz and P. V. Roberts, *Environ. Sci. Technol.*, 1986, **20**, 830.