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Determination of Organic Chemicals in Human Whole Blood: Preliminary Method Development for Volatile Organics

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Extensive commercial, industrial, and domestic use of volatile organic chemicals (compounds with boiling points less than 150°C), virtually assures that the general population will be exposed to some level of this class of chemicals. Sources of volatile organics include dry-cleaning establishments, gasoline stations, household products, and industrial emissions of solvents. Exposure may result from using these services or products or from working in these environments. Also, personal hobbies such as furniture refinishing and model-airplane building may result in exposure. The toxicological effects of high-dose exposure, such as in a work environment, have been determined for a number of volatiles. However, the toxicological effects of low-level exposure, such as may be assumed for the average homeowner or hobbyist, are as yet undetermined for most of these chemicals.

If the general population shows a trend of increasing concentration of a particular chemical, then regulation, decreased usage, or increased worker/consumer protection with respect to that chemical or its precursor may be necessary.

Because blood interacts with the respiratory system and is a major component of the body, it is likely that the analysis of blood will show exposure to volatile organics. Monitoring of the blood in conjunction with monitoring of xenobiotic levels in urine and adipose tissue is an effective way to assess the total body burden resulting from exposure to a chemical. Most published attempts to characterize the levels of aromatic and halogenated volatiles in blood have been conducted in the serum phase of blood (Balkon and Leary 1979, Peoples et al. 1979, Pfaffenberger et al. 1980, Michael et al. 1980). Analysis of plasma has also been conducted (Dowty et al. 1975a, b).

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The problem of determining volatiles that may partition into either the serum/plasma or red-blood-cell phases of the blood, however, has not been fully addressed. Analyzing whole blood can circumvent this problem, but only a few published studies have been made with whole blood (Antoine et al. 1986, Barkley et al. 1980, USEPA 1982) because of the difficulties associated with analyzing a matrix with high protein levels. These difficulties include the binding of the analytes to the proteins, which results in reduced compound recoveries, and the foaming of the samples during purging, which can result in the deposition of blood in the analytical trap. Many of the methods for whole blood employ a heated purge to increase purging efficiencies. Others use antifoam agents to reduce foaming of the blood during purging. The previously mentioned studies, however, have not reported a method designed to detect and confirm parts-per-trillion levels of multiple analytes and also included validation data for the method.

This article introduces a method for the detection and confirmation of selected volatile organics at parts-per-trillion (ppt) levels in whole human blood. Intended for routine use, the method consists of a dynamic headspace purge of water-diluted blood where a carrier gas sweeps the surface of the sample and removes a quantifiable amount of the volatile organics from the blood and into an adsorbent trap. The organics are thermally desorbed from the adsorbent trap and onto the analytical column in a gas-chromatographic/mass-spectrometric (GC/MS) system where limited mass-scan data are taken for qualitative and quantitative identification. The method can be employed for compounds normally defined as volatile organics, such as those on the EPA priority-pollutant-volatiles list. Method validation results and limited population-survey results are also presented here.

MATERIALS AND METHODS

Blood samples for qualitative studies were collected during a blood-donation drive. Two vials of blood of approximately 5 mL each were collected from each of the 22 donors by the attending nurse. The blood flowed from the donor's arm through the blood collection tubing into vials containing 200 μL of a volatile-free EDTA solution (50 mg/mL). The vials were stored at 4°C until analysis. This method of collection was used because it was compatible with normal blood-center operations.

The analytical method for blood is based in part on a previous method developed at Midwest Research Institute for the determination of volatile organics in sludge (MRI 1982). In the analytical method of this paper, a purge vessel designed for dynamic headspace purging was placed in line with the purge-and-trap apparatus on a Finnigan 4500 mass spectrometer. Prior to the analysis, 10 mL of reagent water was added to the vessel, and the water was purged for approximately 15 min before the start of the GC/MS run. This ensured a volatile-free system to which the blood was added.

Individual blood samples were composited to obtain a lower detection limit for the overall composited sample. Seven 5-mL samples were added to the 10 mL of purged water in the purge vessel to form a 35-mL blood composite (45 mL total volume). The mixture was fortified at a 500-ppt (17.5 ng/mL) level with each of seven internal standards. Internal standards were: chloroform-d (Aldrich Chemical Company, Inc., 99%), 1,1-dichloroethane-d_3, 1,1,1-trichloroethane-d_3, 1,2-dichloropropane-d_3, benzene-d_6, 1,1,2,2-tetrachloroethane-d_3, and 1,3-dichlorobenzene-d_4 (Merck Sharp and Dohme Isotopes, 98%).

The headspace of the sample was purged with nitrogen for 10 min at 40 mL/min (400-mL purge volume) at room temperature. The solution was stirred rapidly during the purge. At the end of the purge time the sampling valve was switched to the desorb position, the Tenax TA trap (stainless steel, 250 x 2.64 mm ID) was electrically heated to 180°C, and the volatile contents were desorbed onto the analytical column (1.8 m x 2 mm ID glass packed with 1% Supelco SP-1000/Carbopack B) held at 45°C. The analytes were chromatographed by using a temperature program of 45°C (3-min hold) to 220°C at 8°C/min. Data acquisition began at the moment of desorption.

Full-scan GC/MS data were obtained to identify volatile organic compounds in the composited blood samples. Then, once a list of compounds was established, method validation studies using limited mass scanning (LMS) were employed to obtain better sensitivity for the target compounds. LMS is a technique which involves scanning for a smaller number of ions than in full-scan GC/MS, but more than that normally associated with selective-ion-monitoring (SIM). The compounds in the target list included the EPA volatile priority-pollutants (except the four gases and 2-chloroethyl vinyl ether), styrene, and xylene isomers.

The method was validated by analyzing 12 35-mL composites derived from a 500-mL pool of fresh blood which had been collected from 10 volunteers. Each composite was fortified with the seven internal standards at a level of 500 ppt (17.5 ng/35-mL composite). Analytes were added at the 100-ppt (four replicates), 500-ppt (duplicate), and 1-ppb levels (duplicate). Four replicates of unfortified blood were also analyzed.

RESULTS AND DISCUSSION

In an in-house survey, 5-mL blood samples were collected in duplicate during a routine blood-donation drive, and combined to form three composites with corresponding duplicates. The composites were screened for each of the target analytes and for other unknowns. The results are given in Table 1 and were calculated versus standards purged from water.

Table 1. Analysis results for composited blood samples

Compound	Level detected (ppt)					
	1[a]		2[b]		3[c]	
	Limited mass scan	Full scan	Limited mass scan	Full scan	Limited mass scan	Full scan
Benzene	230	300	34	60	50	60
1,4-Dichlorobenzene	93	80	280	400	29	70
Ethylbenzene	ND[d]	ND	34	ND	ND	ND
m-Xylene	20	ND	10	ND	20	ND
Tetrachloroethene	71	60	96	40	50	300
Toluene	86	300	180	300	180	200

[a]Composite 1 formed from blood of laboratory personnel with
[b]potentially high level of exposure to solvents.
[b]Composite 2 formed from laboratory personnel with potentially
[c]low level exposure.
[c]Composite 3 formed from non-laboratory personnel.
[d]No signal detected.

All analytes detected were below a level of 1 ppb (1 ng/mL composite). Some analytes (e.g., m-xylene and ethylbenzene) were detected by LMS but not by full-scan GC/MS due to the inherent difference in sensitivity between the two methods. In general, however, there was good agreement between the full-scan and limited-mass scan. Dichloromethane was detected in all water blanks, blood blanks, and blood samples. The extreme sensitivity of the LMS method and the prevalent use of dichloromethane in our laboratories may explain its detection. No data for dichloromethane could be confidently reported for these composites because the source could not be established. Chloroform was detected in all samples at trace levels (< 20 pg/mL composite). No other compounds in the analyte list were detected. The estimated detection limits ranged from 20 to 500 ppt (pg/mL composite) for LMS detection and 200 to 5,000 ppt for full-scan detection. The majority of peaks were present at such low intensities that they could not be identified because of incomplete spectra and high background relative to the peak intensity. Acetone was present at relatively high levels, however, and was identified in all blood samples.

The method validation results were calculated by two methods. In the first method, absolute recoveries of the analytes in the blood samples were determined versus their responses in the aqueous standards by the external standard technique. In the second method, analyte recoveries relative to internal standard responses were calculated from updated relative response factors (RRF) determined from the calibration curve established at the beginning of the analysis.

The results of the method validation are given in Table 2 for the compounds for which a deuterated internal standard existed.

While the absolute recoveries for these compounds were low, the use of a deuterated analog to adjust for the low purging efficiencies by the internal standard technique proved relatively accurate for all the compounds tested. Precision was generally improved by using the internal standard versus the external standard technique and was approximately 30% (RSD) overall compounds shown in Table 2. Since only duplicates were analyzed at the 500-ppt and 1-ppb levels, however, outliers at these levels could not be determined. The use of the internal standards to correct for the depressed purging efficiencies of other compounds (not structurally related to the internal standards) did not prove accurate since their respective absolute recoveries could change relative to one another from one analysis to another.

Many methods designed to recover volatile analytes from biological samples (urine, serum, adipose) use a heated purge to increase absolute recoveries of the volatiles from the matrix (Barkley et al. 1980, Radzikowska-Kintzi and Jakubowski 1981). This was avoided in the present study for two reasons. One was to avoid deposition of water into the ion source of the mass spectrometer (or having to add devices to the purge equipment to remove such water). The second was to avoid the heat-related transformation of chemicals in the blood to detectable volatiles, such as in the conversion of trichloroacetic acid to chloroform (Peoples 1979). Without elevating the temperature of the blood during the purging step, however, the absolute recoveries of the late eluting volatiles were low.

Further experiments are anticipated with a less rigorously heated purge than previously reported (Peoples 1979) and with blood diluted with different volumes of water when analyzed to determine if increasing the absolute recoveries of the late-eluting volatiles will aid in their quantitation by the internal standard method. In addition, validation data for the remaining target analytes will be obtained in forthcoming evaluations by using additional labeled analogs. Capillary GC/MS may also be investigated as an alternative to the packed column approach currently in use.

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Table 2. Method validation results

Compounds	Analyte concentration in unfortified blood (ppt)		Analyte recovery (%) in fortified blood (100 ppt)[a]		Analyte recovery (%) in fortified blood (500 ppt)[a]		Analyte recovery (%) in fortified blood (1 ppb)[a]	
	Average (n = 4)	RSD[b]	Average (n = 4)	RSD	Duplicates		Duplicates	
Benzene	33[c]	78	85	27	48	65	57	56
	120[d]	25	120	57	160	100	200	110
Chloroform	ND[e]	-	37	41	36	59	49	46
	16	117	120	42	130	100	150	95
1,3-Dichlorobenzene	ND	-	ND	-	4	3	6	7
	ND	-	120	25	150	94	120	110
1,1-Dichloroethane	ND	-	76	15	48	61	60	55
	28	11	110	17	100	80	120	90
1,2-Dichloropropane	ND	-	71	13	43	57	56	48
	51	38	140	34	160	94	170	91
1,1,2,2-Tetrachloroethane	ND	-	ND	-	14	33	27	22
	ND	-	ND	-	110	140	120	87
1,1,1-Trichloroethane	53	51	110	62	31	100	50	53
	150	12	160	41	130	140	170	95

[a] Blank corrected.
[b] Relative standard deviation.
[c] Level determined by the external standard method (absolute recovery or level).
[d] Values determined by the internal standard method (adjusted).
[e] Not detected. ND is approximately < 100 ppt for all compounds except 1,1,2,2-tetrachloroethane, which is < 500 ppt. For the purpose of obtaining an average, NDs were given the value of zero where appropriate.

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