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Determination of Chlorobenzenes in Air and Biological Samples by Gas Chromatography with Photoionization Detection

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Methods are described for the determination of chlorobenzenes (monochlorobenzene through hexachlorobenzene) at parts-per-billion levels in air and human urine and blood samples by gas chromatography with photoionization detection. Prior to analysis, chlorobenzenes in air are collected/concentrated on XAD-2 resin, and solvent desorbed. The method for urine and blood samples consists of carbon tetrachloride extractions, silica gel column chromatography and concentration with a Kuderna-Danish concentrator. A specially designed column packing allows separation of the chlorobenzenes in 16 min. Data presented indicate that collection and desorption efficiencies for chlorobenzenes with concentrations between 5 ppb (v/v) and 15 ppm (v/v) in air to be 95 ± 12% (2σ). Chlorobenzene recoveries from urine and blood samples are 83 ± 12% for concentrations between 1 and 500 ppb (ng/g).

Millions of pounds of chlorobenzenes are produced annually by chemical companies in the United States. These chlorinated benzenes are used as process solvents, starting materials, or intermediates for the manufacture of other chemical products such as pesticides, phenols, dyestuffs, etc. The environmental impact and toxicology of chlorobenzenes have increasingly been of interest. Reports have documented ecology problems stemming from hexachlorobenzene use or misuse (*1–4*) and have indicated chronic toxicity from exposure to hexachlorobenzenes. Toxicity and feeding studies have been completed with rats, rabbits, monkeys, pigs, and fish (*5–11*). Extensive studies have been conducted dealing with metabolism, metabolite identification, pharmacokinetics, and clearance rates of chlorobenzenes after ingestion or exposure (*12–27*). The vast majority of the environmental studies, toxicity studies, and analytical methodology have dealt with *hexachlorobenzene* and related pesticides determined by gas chromatography with electron capture detection (*28, 29*).

This paper emphasizes improved analytical methodology to encompass determinations of all the chlorobenzenes (monochlorobenzene through hexachlorobenzene) in environmental and biological sample types—air, urine, and blood. By using a gas chromatograph equipped with a photoionization detector, a designed GC column packing, and appropriate sample preparation and concentration techniques, all chlorobenzenes can be determined in environmental and biological samples at low parts-per-billion levels. This methodology allows separation and quantitation of monochlorobenzene, 1,2-, 1,3-, and 1,4-dichlorobenzenes, 1,2,3-, 1,2,4-, and 1,3,5-trichlorobenzenes, 1,2,3,4- and 1,2,4,5-tetrachlorobenzenes, pentachlorobenzene, and hexachlorobenzene with a single 16-min chromatogram. Before application of this methodology for environmental studies, the methods were validated to define accuracy, precision, and minimum detection limits.

EXPERIMENTAL

Reagents. *Amberlite XAD-2 Resin as an Air Sampling Adsorbent.* Amberlite XAD-2 resin is a synthetic insoluble cross-linked polystyrene polymer in the form of 20–50 mesh beads produced by Rohm and Haas Co. Purified XAD-2 resin is available from Applied Science Laboratories, Inc. Prior to use, it was necessary to further clean up the resin and dry it enough for packing and unpacking air sampling tubes.

The XAD-2 resin was placed in a bottle and extracted twice with carbon tetrachloride. The resin was then filtered and washed with methanol and then water. This hydrates the resin. The resin was then dried in a Petri dish in an oven at 100–110 °C for less than 30 min, allowing the resin to dry out only enough to prevent clumping in air sampling tubes. (Should complete dehydration of the resin occur, optimum adsorption results may not be attained.)

Silica Gel for Column Clean-Up of Urine or Blood Samples. Chromatographic grade silicic acid as 100–200 mesh Bio-Sil A (Bio-Rad Laboratories, Inc., Richmond, Calif. 94804) was used for clean-up of urine and blood samples (separation of chlorobenzenes from the bulk of the sample matrix). Before use, the adsorbent was activated (dried) in an oven at 180 °C for 16 h. The adsorbent was cooled and capped (Poly-Seal) in a glass bottle for storage in a desiccator before use. If reagent blanks show chlorobenzene contamination, the silica gel may be Soxhlet extracted with carbon tetrachloride before activation in the oven.

Gas Chromatographic Column Packings. Three gas chromatographic column packings can be employed for these analyses. Column packing (A), 0.50% Carbowax E-20M over bonded E-20M on 80–100 mesh Chromosorb W-AW + 5% Synerg C, is a specially prepared packing available from H-NU Systems, Inc. Its design provides high efficiency, high solvent capacity, isomer resolution characteristics, and minimum liquid phase bleed. This packing was packed into a 10 ft × $^1/_8$ in. silanized glass column.

(B) A faster gas chromatographic column packing was also developed, 0.20% Carbowax E-40M + 0.50% Synerg C on 130–140 mesh GLC-110. Its design characteristics are essentially the same as those described for column packing A. It too, is available from H-NU Systems. However, this packing is a surface coated glass bead packing and provides certain unique performance characteristics especially compatible with high sensitivity determinations using the photoionization detector. In particular, the

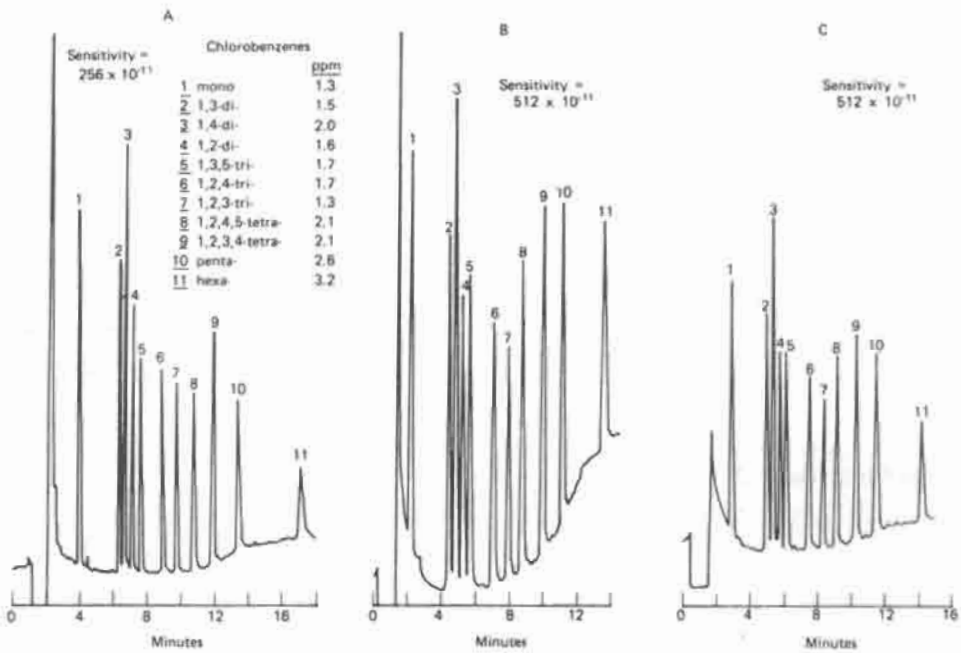

Figure 1. Comparison of high performance column packings for the separation of chlorobenzenes

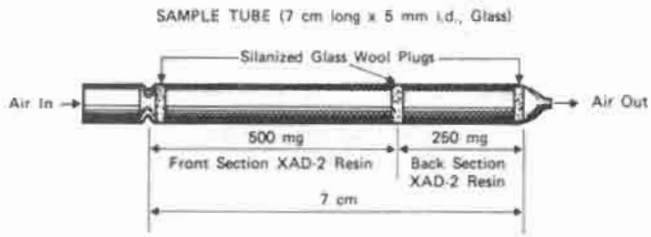

Figure 2. Air sampling tube containing XAD-2 adsorbent

optimum carrier velocity (v_{opt}) for this packing, as determined from the Van Deemter plot for hydrocarbon elution, is unusually low for 2-mm i.d. columns. This particular packing demonstrates a v_{opt} of approximately 8 cm^3/minute for helium carrier when tested in a 2 mm i.d. × 180 cm glass column. This combination of high capacity, high efficiency, short retention times, and low carrier gas flow rates results in improved instrumental sensitivity when used with the photoionization detector. (For the photoionization detector, sensitivity is inversely related to carrier gas flow rate.) This packing was packed into a 6 ft × $^1/_8$ in. silanized glass column. The resulting column packing gave an extremely fast efficient separation of chlorobenzenes. However, at very low levels, monochlorobenzene was lost in the solvent peak. (The improved sensitivity obtained by lowering the carrier flow rate also affected the solvent peak.) As a result, a third column was packed.

(C) This column was a combination of the first two column packings. The first 9 in. of column B described above were unpacked and replaced with the packing described for column A. This front end packing moved the monochlorobenzene peak away from the solvent front and protected the packing B from stripping of the liquid phase during injection.

The chromatographic separations obtained with these three columns are compared in Figure 1. Notice the improved sensitivity obtained with columns B and C. These columns completely separate all chlorobenzene isomers except 1,2,3,5-tetrachlorobenzene which is not completely resolved from 1,2,4,5-tetrachlorobenzene. However, the 1,2,3,5-tetrachlorobenzene isomer is usually not found in environmental and biological samples.

Procedure for Air Samples. The air sampling tube used to adsorb/concentrate chlorobenzenes is shown in Figure 2. Collection tubes were packed with two sections of Amberlite XAD-2 resin separated by a silanized glass wool plug. Flow rates of 100–200 mL/min were used to pull air through the tube collecting chlorobenzenes. For area and personnel sampling, 4-h samples were collected to provide a time-weighted average (TWA) of the exposure.

Following sample collection, each section of adsorbent in the air collection tube was desorbed with 5 mL of carbon tetrachloride shaken for 20 min on a mechanical shaker, and analyzed by gas chromatography by using the following conditions:

Instrument:	Varian 2700 Gas Chromatograph	
Column:	(column A, described previously)	
Detector:	H-NU Systems, Inc., photoionization detector with 10.2-eV lamp	
Temperatures:	Column:	50 (3-min hold) → 200 °C (6-min hold) at 10 °C/min
	Detector:	250 °C
	Injection Port:	150 °C
Flow:	Carrier:	nitrogen at 18 mL/min
Injection Volume:	5 μL	
Solvent:	CCl_4	

Procedure for Urine Samples. Urine samples should be collected and stored in glass jars. A 20-g sample was weighed into a vial and extracted three times with 3 mL of carbon tetrachloride. The carbon tetrachloride extracts were combined and passed through a silica gel column to further clean up the sample. The silica gel column used is shown in Figure 3. The 9 mL of extract was added to the top of the column along with a small volume of carbon tetrachloride used to wash the vial. The eluate was collected in a vial and additional CCl_4 (approximately 8 mL) was added to the top of the column until a total of 13 mL of eluate was collected. This 13 mL contained the chlorobenzenes, but no longer contained interferences from the sample matrix.

The sample was concentrated from 13 to 1 mL in a Kuderna-Danish evaporator at 180 °C. Then the concentrated sample was analyzed by gas chromatography with a photoionization detector (GC-PID). The GC conditions are listed below:

Instrument:	Varian 2700 Gas Chromatograph
Column:	6 ft × $^1/_8$ in, glass (column C, described previously)
Detector:	H-NU Systems, Inc, photoionization detector with 10.2-eV lamp
Temperatures:	Column: 60 (2-min hold) → 190 °C at 12 °C/min
	Detector: 250 °C
	Injection Port: 175 °C
Flow:	Carrier nitrogen at 8 mL/min
Injection Volume:	5 μL
Solvent:	CCl_4

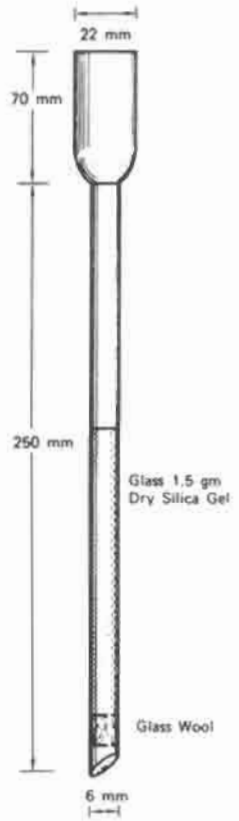

Figure 3. Column for clean-up of urine and blood samples

A typical standard chromatogram is shown in Figure 1C.

Procedure for Blood Samples. Blood samples should be collected and stored in glass tubes. The samples should be frozen during storage and should contain an anticoagulant. Both heparin and EDTA were used as anticoagulants in these experiments with no differences in results. For blood samples, a 5-g sample was weighed into a vial and diluted with 30 mL of deionized Milli-Q water. The sample was then extracted three times with 3 mL of carbon tetrachloride. The samples were cleaned up, concentrated, and analyzed by using the same procedures described for urine samples.

RESULTS AND DISCUSSION

A method is described for the solid adsorbent collection, solvent desorption, and gas chromatographic determination of chlorobenzenes in air. The procedure is designed for ambient air sampling and Industrial Hygiene area and personnel monitoring. Another method is detailed for the determination of all the chlorobenzenes in urine and whole blood samples. The use of urine and blood samples as indicators of occupational exposure is gaining increased attention because of its ability to provide a link between Industrial Hygiene surveys of the work environment and Medical Health Inventory surveys screening employee health.

Table I shows minimum detection limits (MDLs) and threshold limit values for chlorobenzenes in air samples. Validation data are detailed in Tables II and III for simulated air samples. The data presented indicate that collection and desorption efficiencies for the chlorobenzenes with concentrations between 5 ppb (v/v) and 15 ppm (v/v) in air to be approximately 95%. The precision of the procedure is ±12% (2σ). The 4-h collection limit for monochlorobenzene appears to be approximately 4 ppm (v/v) at 150 mL/min when similar concentrations of other chlorobenzenes are present. Under these conditions, greater than 15% of the total monochlorobenzene concentration was observed in the back section. Insignificant amounts (less than 5%) of the other chlorobenzenes were observed in the back section when concentrations up to 15 ppm (v/v) were collected for 4 h. If monochlorobenzene concentrations are greater than 4 ppm (v/v) or other chlorobenzene concentrations are greater than 15 ppm (v/v), breakthrough may occur. In these cases, consecutive sample tubes, shorter sampling times, or slower flow rates may provide adequate collection, but further validation data should be required.

Table I. Minimum Detection Limits (MDLs) and Threshold Limit Values (TLVs) for Chlorobenzenes in Air

A. Minimum Detection Limits (Using Column A)

compound	MDLs in ppb (ng/mL)	MDLs in ppb (v/v) for 24-L air sample, 5 mL desorption vol
monochlorobenzene	15	0.7
dichlorobenzenes	20	0.7
trichlorobenzenes	30	0.8
tetrachlorobenzenes	35	0.8
pentachlorobenzene	45	0.9
hexachlorobenzene	70	1.2

B. Threshold Limit Values (TLVs) and Industrial Hygiene Guides (IHGs)

compound	TLV[a]	IHG[b]
monochlorobenzene	75 ppm (v/v)	75 ppm (v/v)
1,2-dichlorobenzene	50 ppm (v/v)	50 ppm (v/v)
1,3-dichlorobenzene	-	-
1,4-dichlorobenzene	75 ppm (v/v)	75 ppm (v/v)
1,2,3-trichlorobenzene	-	-
1,2,4-trichlorobenzene	-	5 ppm (v/v)
1,3,5-trichlorobenzene	-	-
1,2,3,4-tetrachlorobenzene	-	0.4 ppm (v/v)
1,2,3,5-tetrachlorobenzene	-	-
1,2,4,5-tetrachlorobenzene	-	0.4 ppm (v/v)
pentachlorobenzene	-	0.1 ppm (v/v)
hexachlorobenzene	-	0.06 ppm (v/v) or 0.7 mg/m³

[a] Established by American Conference of Governmental Industrial Hygienists. [b] Established and used by the Dow Chemical Co.

Table III shows data collected to determine the effects of high air humidity and air collection tube storage on chlorobenzene recoveries. Samples collected at 80% humidity still showed 96% recoveries and samples stored without refrigeration for 35 days showed only slightly lower recoveries. The combined effects of high humidity and sample tube storage also showed no significant chlorobenzene losses.

Data from phase equilibrium experiments are shown in Table IV. These data show that an equilibrium exists for the distribution of chlorobenzenes between the adsorbed phase (XAD-2 resin) and the solvent phase (CCl_4). Phase equilibrium experiments showed 96% recovery of chlorobenzenes is obtained by using 500 mg of XAD-2 resin and 5 mL of CCl_4.

Data for the determination of chlorobenzenes in urine and blood are shown in Tables V–VII. No chlorobenzenes were detected in reagent blanks, seven control urine samples, or seven control blood samples with detection limits shown in Table V. Minimum detection limits ranged from 0.8 to 6 ppb (ng/g of sample) for urine samples and 3 to 20 ppb for blood samples. Detection limits were relatively high for monochlorobenzene because it elutes close to the solvent peak and is lost in the solvent peak at high sensitivity (using column C). Figure 4 shows typical chromatograms of reagent blanks and control samples.

Data to define recoveries, accuracy, and precision are shown in Tables VI and VII. Recoveries of chlorobenzenes from

Table II. Recovery Data for Chlorobenzenes in Air[a]

no.	av conc[c] Cl_x-benzene in air, ppb (v/v)	% recovery of chlorobenzenes[b,d] mono-	1,2-di-	1,3-di-	1,4-di-	1,2,3-tri-	1,2,4-tri-	1,3,5-tri-	1,2,3,4-tetra-	1,2,4,5-tetra-	penta-	hexa-	mean recovery ± SD
1	5	101	60	-	86	89	84	97	92	99	89	99	90 ± 12
2	40	100	92	102	95	100	82	98	94	95	95	105	96 ± 6
3	110	86	99	100	102	98	99	103	91	106	95	85	97 ± 7
4	150	100	93	98	92	101	82	96	95	96	94	94	95 ± 5
5	230	96	98	100	101	93	99	102	93	104	99	92	98 ± 4
6	240	99	97	99	95	107	84	97	98	99	95	93	97 ± 6
7	440	99	100	99	90	95	102	91	106	102	100	98	98 ± 5
8	480	100	102	105	100	106	89	98	104	101	100	101	100 ± 5
9	570	99	93	94	86	88	95	86	96	109	92	90	93 ± 7
10	790	107	103	105	101	101	92	99	100	99	102	99	101 ± 4
11	960	100	93	98	92	101	82	96	95	96	94	94	95 ± 5
12	2900	91	99	97	99	99	100	104	103	96	95	89	97 ± 5
13	3100	86 (15)	96 (10)	94 (10)	94 (10)	92	89	97	102	95	101	97	95 ± 5
14	8300	72 (27)	87 (14)	88 (17)	92 (9)	92	97	96	99	97	101	95	92 ± 8
15	8700	42 (15)	100	98	98	98	99	101	100	100	95	95	93 ± 17
16	15000	15 (44)	92 (2)	88 (3)	92 (2)	96	94	96	95	92	94	94	86 ± 24
													av: 95 ± 8

[a] Chlorobenzene recoveries from direct injection onto front of XAD-2 resin tube with 20-40 L of air pulled at 100-250 mL/min. [b] Percent recoveries are listed for front section of air sampling tube only, except when number is in parentheses to designate percentage found on back tube section. [c] Average concentration chlorobenzenes is the average of monochlorobenzene through hexachlorobenzene concentration in air simulated. [d] These experiments were conducted at ambient humidity.

Table III. Recovery Data for Air Samples: Simulated Air Samples Showing Humidity and Storage Effects[a-d]

no.	av Cl_x-benzene concn in air, ppb (v/v)	% recovery mono-	1,2-di-	1,3-di-	1,4-di-	1,2,3-tri-	1,2,4-tri-	1,3,5-tri-	1,2,3,4-tetra-	1,2,4,5-tetra-	penta-	hexa	mean recovery ± SD
(a) Storage													
1	540	81	88	99	94	102	91	96	92	93	100	100	94 ± 6
2	580	79	81	91	95	96	85	89	89	89	95	96	89 ± 6
(b) Humidity													
3	160	102	101	106	101	106	87	102	98	100	99	93	99 ± 5
4	1300	103	89	90	81	88	93	82	96	106	96	93	92 ± 8
5	140	91	85	94	91	107	86	93	88	89	97	106	93 ± 7
(c) Combined Storage and Humidity													
6	4400	67 (23)	85	84	84	86	85	86	84	85	88	88	77 ± 24
7	85	90	80	94	90	102	83	88	86	90	93	103	91 ± 7
8	160	90	80	95	90	102	86	91	92	92	95	100	92 ± 6
9	790	78 (14)	93	93	89	98	86	90	91	92	96	98	91 ± 6

[a] Sample tubes 1 and 2 were stored for 35 days without refrigeration before analysis to check stability of Cl_x-benzenes on XAD-2 resin tubes. [b] Samples 3 and 4 were prepared with 80% humidity air. Eighty percent relative humidity is obtained by passing air through an impinger containing a saturated solution of $(NH_4)_2SO_4$ in H_2O before the air enters the sample tube. [c] Samples 5-9 were prepared with 80% humidity air and stored for 2-35 days before analysis. [d] Recoveries are for front sections of tube only, except when number in parentheses shows recoveries from tube back sections.

Table IV. Phase Equilibrium Data: Percent Recoveries of Chlorobenzenes

	wt XAD-2 resin, mg	vol of CCl_4 solvent, mL	av concn,[a] ppb (ng/mL)	% recovery[b]
A. vary resin (XAD-2) wt;	250	5	180	98 ± 7
constant solvent;	500	5	180	96 ± 7
constant chlorobenzenes concn	1000	5	180	87 ± 11
	1500	5	180	79 ± 15
B. vary solvent (CCl_4) vol;	500	2	180	87 ± 10
constant resin wt;	500	5	180	96 ± 7
constant chlorobenzenes concn	500	10	180	97 ± 7
	500	15	180	101 ± 7
C. vary chlorobenzenes concn;	500	5	90	99 ± 13
constant resin wt;	500	5	180	96 ± 7
constant solvent vol	500	5	119000	99 ± 1

[a] Average concentration is the average concentration of monochlorobenzene through hexachlorobenzene in the sample.
[b] Percent recovery is the average of all chlorobenzenes recoveries.

Table V. Minimum Detection Limits (MDLs) for Chlorobenzenes in Urine and Blood Samples (Using Column C)

	minimum detection limit (chlorobenzenes)										
units	mono-	1,3-di-	1,4-di-	1,2-di-	1,3,5-tri-	1,2,4-tri-	1,2,3-tri-	1,2,4,5-tetra-	1,2;3,4-tetra-	penta-	hexa-
in ng/mL of solvent	97	14	15	18	22	23	20	26	23	36	82
in ng/g of urine	4.9	0.70	0.75	0.90	1.1	1.2	1.0	1.3	1.2	1.8	4.1
in ng/g of blood	19	2.8	3.0	3.6	4.4	4.6	4.0	5.2	4.6	7.2	16
	With 83% Recoveries										
in ng/g of urine	5.9	0.84	0.90	1.1	1.3	1.4	1.2	1.6	1.4	2.2	4.9
in ng/g of blood	23	3.4	3.6	4.3	5.3	5.5	4.8	6.3	5.5	8.7	19

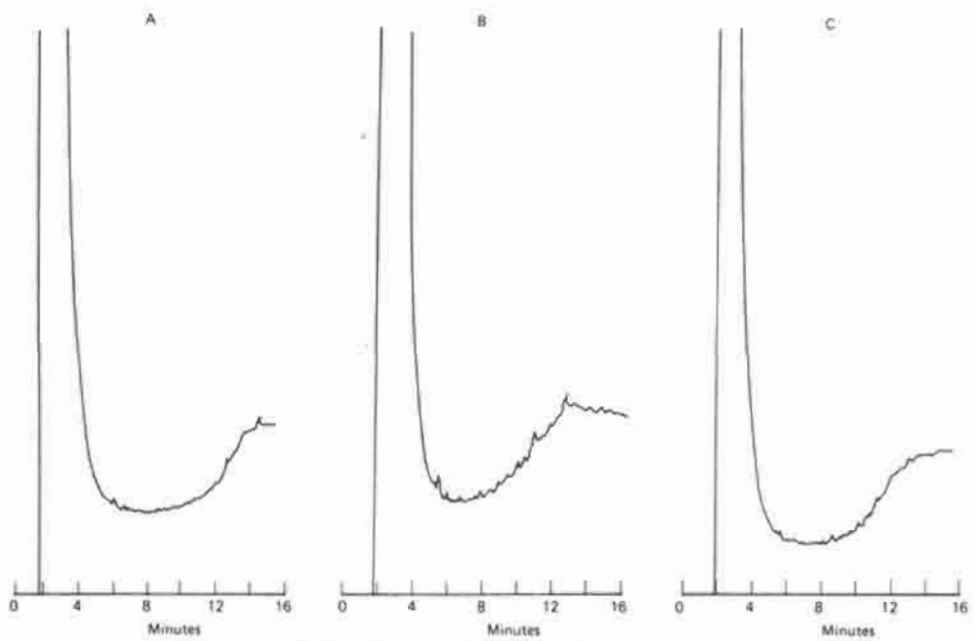

Figure 4. Typical chromatograms for (A) reagent blanks, (B) control urine samples, and (C) control blood samples

spiked urine and blood samples averaged 83 ± 12% (2σ). Typical chromatograms of a spiked blood sample and standard are shown in Figure 5.

Analytical Considerations. *Detector Linearity.* The photoionization detector exhibits a linear response for chlorobenzenes with concentrations covering at least five orders of magnitude (20 ng/mL to 200 μg/mL). In addition, the photoionization detector response for a given molar concentration of any chlorobenzene is identical and is linearly related to molar concentration.

Best Solvent for Preparation of Urine and Blood Samples. Solvents chosen for the preparation of urine and blood samples must effectively extract chlorobenzenes from the sample matrix, elute chlorobenzenes from the silica gel clean-up column, recover chlorobenzenes during concentration with a Kuderna-Danish evaporator, and be compatible with the photoionization detector. An important feature of this analysis was the ability to use carbon tetrachloride as the solvent for

Table VI. Recovery Data: Determination of Chlorobenzenes in Urine

no.	sample wt, g	concn[a] chloro-benzenes	% recovery of chlorobenzenes											av recovery
			mono-	1,3-di-	1,4-di-	1,2-di-	1,3,5-tri-	1,2,4-tri-	1,2,3-tri-	1,2,4,5-tetra-	1,2,3,4-tetra-	penta-	hexa-	
1	19.99	v		82	87	94	82	87	98	91	89	80	73	86 ± 7
2	20.00	v		94	97	95	98	96	98	91	96	95	91	95 ± 3
3	19.99	w		75	85	87	82	87	88	86	85	75	82	83 ± 5
4	20.00	w		87	90	91	90	87	98	95	89	95	91	91 ± 4
5	19.99	w		81	89	83	88	87	83	85	84	87	88	86 ± 3
6	19.98	x		70	76	70	75	80	85	76	82	74	82	77 ± 4
7	19.98	x		81	84	91	82	80	85	91	89	90	91	86 ± 4
8	20.00	x		75	78	81	80	83	83	83	82	88	90	82 ± 4
9	19.99	x		83	82	81	79	87	91	82	90	88	90	85 ± 4
10	20.00	x		75	78	83	81	86	90	82	83	80	79	82 ± 4
11	20.00	x		70	71	77	75	78	82	80	78	78	79	77 ± 4
12	20.01	x		69	74	71	76	77	78	82	82	87	88	78 ± 6
13	19.98	y		70	69	72	70	72	74	70	73	70	72	71 ± 2
14	19.97	y		81	78	81	77	79	82	80	82	78	78	80 ± 2
15	20.00	z	74	83	83	86	85	84	86	83	83	84	84	83 ± 3
16	20.00	z	72	78	79	81	82	82	84	83	85	85	87	82 ± 4
17	20.01	z	72	73	76	78	81	80	82	83	85	85	87	82 ± 4
18	20.00	z	74	86	85	89	87	89	92	88	91	91	90	87 ± 5
19	20.00	z	69	74	75	79	80	78	79	78	80	82	81	78 ± 4
av recovery:			72 ± 2	78 ± 7	81 ± 7	83 ± 7	82 ± 6	83 ± 6	86 ± 7	84 ± 6	85 ± 5	84 ± 7	84 ± 6	83 ± 5

[a] Concentration of chlorobenzenes added to 20-g urine samples:

symbol	concentration added (ng/g sample)											approx relationship to MDLs
	mono-	1,3-di-	1,4-di-	1,2-di-	1,3,5-tri-	1,2,4-tri-	1,2,3-tri-	1,2,4,5-tetra-	1,2,3,4-tetra-	penta-	hexa-	
v	1.3	1.5	2.0	1.6	1.7	1.7	1.3	2.1	2.1	2.6	3.1	1 × MDL
w	2.6	3.0	4.0	3.1	3.5	3.4	2.6	4.2	4.1	5.1	6.3	2 × MDL
x	5.2	6.1	8.1	6.2	7.0	6.8	5.2	8.5	8.2	10	13	4 × MDL
y	13	15	20	16	17	17	13	21	21	26	31	10 × MDL
z	52	61	81	62	70	68	52	85	82	100	130	40 × MDL

[b] Minimum detection limits for urine samples are shown in Table V.

Table VII. Recovery Data: Determination of Chlorobenzenes in Whole Blood

no.	sample wt, g	concn[a] chloro-benzenes added	% recovery											av recovery
			mono-	1,3-di-	1,4-di-	1,2-di-	1,3,5-tri-	1,2,4-tri-	1,2,3-tri-	1,2,4,5-tetra-	1,2,3,4-tetra-	penta-	hexa-	
1	5.00	v		74	85	77	77	72	82	73	70	70	70	75 ± 5
2	4.99	v		93	95	94	95	92	92	95	88	90	90	90 ± 6
3	5.01	v		81	97	83	88	92	89	82	80	80	75	85 ± 7
4	5.00	w		71	79	72	76	82	88	73	70	75	80	77 ± 6
5	5.00	w		85	87	98	95	92	95	93	87	75	80	89 ± 7
6	4.98	w		81	95	83	88	95	94	92	89	93	93	90 ± 5
7	5.00	x		71	85	80	79	79	88	73	71	70	70	77 ± 7
8	5.00	x		89	97	96	95	95	94	85	90	93	85	92 ± 4
9	4.99	x		74	85	81	68	78	80	70	73	70	81	76 ± 6
10	5.00	x		96	95	98	77	95	91	74	75	72	85	86 ± 10
11	4.97	x		78	78	75	70	73	71	74	70	72	72	73 ± 3
12	4.99	x		83	92	90	84	93	94	81	84	88	86	88 ± 5
13	4.99	x		74	80	78	78	77	75	75	78	83	79	78 ± 3
14	5.00	y		97	98	97	96	93	98	86	84	80	80	91 ± 8
15	4.98	y	80	97	98	97	90	89	96	70	81	75	73	87 ± 9
16	5.00	z	72	78	84	77	78	78	79	78	76	73	71	77 ± 4
17	4.98	z	75	85	91	85	85	87	87	84	85	82	84	85 ± 4
18	4.99	z	74	82	87	81	81	81	85	83	82	82	81	82 ± 3
19	5.00	z	66	69	84	70	80	80	81	70	77	77	81	78 ± 5
20	5.00	z	72	79	79	83	78	77	75	77	77	80	81	78 ± 3
av recovery:			73 ± 5	82 ± 8	89 ± 7	85 ± 8	83 ± 8	88 ± 7	80 ± 7	80 ± 7	80 ± 7	79 ± 7	79 ± 6	83 ± 6

[a] Concentration of chlorobenzenes added to 5-g blood samples:

symbol	concn added, ng/g of blood											approx relationship to MDLs
	mono-	1,3-di-	1,4-di-	1,2-di-	1,3,5-tri-	1,2,4-tri-	1,2,3-tri-	1,2,4,5-tetra-	1,2,3,4-tetra-	penta-	hexa-	
v	5.2	6.1	8.1	6.2	7.0	6.8	5.2	8.5	8.2	10	13	1 × MDL
w	10	12	16	12	14	14	10	17	16	21	25	2 × MDL
x	21	24	32	25	28	27	21	34	33	41	50	4 × MDL
y	52	61	81	62	70	68	52	85	82	100	130	10 × MDL
z	210	240	320	250	280	270	210	340	330	410	500	40 × MDL

[b] Minimum detection limits for blood samples shown in Table V.

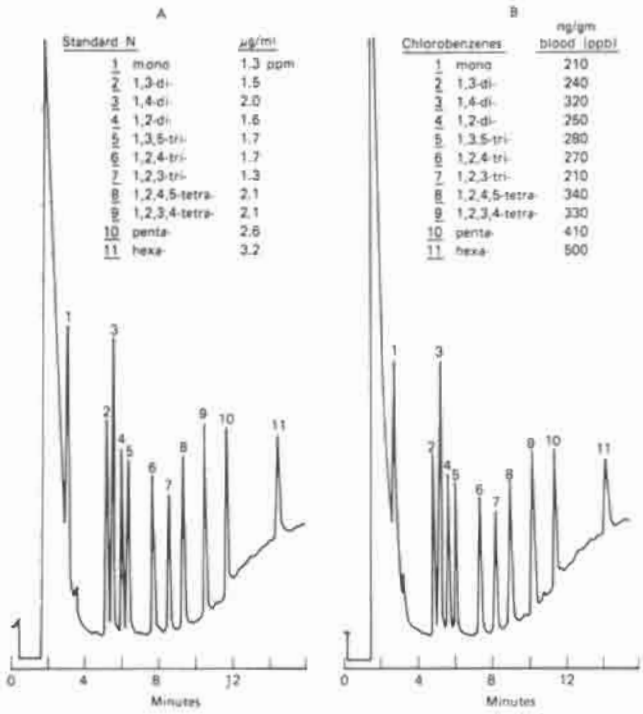

Figure 5. Typical chromatograms for (A) standard and (B) fortified (spiked) blood sample (sample no. 18, Table VII)

all these steps in the procedure. This eliminated the need for solvent exchanges and optimized recoveries. Experiments were also carried out with hexane and methylene chloride. However, hexane was not compatible with the photoionization detector and methylene chloride extracts more impurities from the sample matrix. Extraction of chlorobenzenes from urine and blood was accomplished with CCl_4 because this solvent efficiently extracts chlorobenzenes, does not form stable emulsions, and extracts very little of the urine or blood matrices which might interfere with the determination. The samples were best extracted without changing pH.

Concentrating the sample without losses of lower boiling chlorobenzenes is difficult. As a result, the samples were concentrated only one time during the sample preparation—immediately prior to analysis. Samples cannot be concentrated by evaporation under a stream of prepurified nitrogen without significant losses of lower boiling chlorobenzenes. By using a Kuderna-Danish concentrator with tubes and three-stage Snyder columns, samples can be efficiently concentrated *if* the chlorobenzenes are in a suitable solvent and if the temperature of the heater does not exceed 180 °C. For the concentration of chlorobenzene solutions from 13 to 1 mL, only CCl_4 allowed good recovery of chlorobenzenes. Losses of chlorobenzenes from methylene chloride and hexane were significant unless 1 mL of CCl_4 was added to the sample before concentration.

This procedure is not intended to determine chlorobenzenes which have been metabolized or biologically conjugated with other molecules. It is simply applicable to the determination of free chlorobenzenes in urine and blood. The analytical procedure described will provide reliable data for the determination of chlorobenzenes in human urine and blood samples and air samples without the use of sophisticated instrumentation.

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