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Note

Quantitative gas chromatographic determination of hexachloro-1,3-butadiene in whole rat blood at part per trillion levels

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1,1,2,3,4,4-Hexachloro-1,3-butadiene, (HCBD), is a colorless liquid industrial by-product found in the manufacturing of chlorinated hydrocarbons. The toxicity associated with chronic exposure of laboratory rats has been reported[1].

In order to assess the pharmacokinetics of HCBD administered to rats an analytical method was required to determine low level concentrations of HCBD in whole rat blood. Previous analytical methods for HCBD in blood required colorimetric or UV spectroscopic detection of an HCBD titration product[2]. This paper describes a simple gas chromatographic (GC) method for the determination of HCBD in whole rat blood at part per trillion* levels.

MATERIALS AND METHODS

Material

HCBD and 1,2,3,4-tetrachlorobenzene (TCB) were supplied by The Dow Chemical Company (Midland, MI, U.S.A.). Distilled-in-glass methanol and UV hexane were purchased from Burdick & Jackson Labs. (Muskegon, MI. U.S.A.). Healthy, adult male Fischer 344 rats were purchased from the Charles River Breeding Laboratories (Wilmington, MA, U.S.A.).

Instrumentation

A Varian 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector and CDS-111 Integrator was used for quantitative analysis of the samples. Separation was obtained on a 2.83 m × 2 mm I.D. glass column containing 3% OV-17 on 100–120 mesh Chromosorb W-HP (Supelco, Bellefonte, PA, U.S.A.). The column was conditioned overnight at 150°C with a nitrogen flow of 20 ml/min. During the analysis the GC column was maintained at 120°C with a nitrogen flow-rate of 20 ml/min. The injection port and detector temperature were 120° and 250°C, respectively.

* Throughout this article the American trillion (10^{12}) and billion (10^9) are meant.

Standards

TCB stock solutions were prepared by adding accurately weighed amounts of TCB to hexane. Two of these stock solutions, 2.96 ng/ml and 3.26 · 10 ng/ml, were used in preparing HCBD working standards and for solvent extraction of HCBD from blood. TCB working standards were prepared by diluting the stock solutions into the calibrated detector range of $9.17 \cdot 10^{-1}$ ng/ml to 5.55 · 10 ng/ml TCB.

HCBD working standards were prepared by adding an accurately measured amount of HCBD to an aliquot of either the 2.96 ng/ml or 3.26 · 10 ng/ml TCB stock solution yielding HCBD concentrations of $1.70 \cdot 10^{-2}$ ng/ml to $1.67 \cdot 10^{2}$ ng/ml. Generally, the 2.96 ng/ml TCB stock solution was used for all HCBD working standards below 2 ng/ml and the 3.26 · 10 ng/ml TCB stock solution was used for all HCBD working standards above 2 ng/ml. This was done to keep both the HCBD and TCB peak heights on scale for peak height measurements if necessary.

HCBD blood spiking solutions were prepared by adding accurately measured amounts of HCBD to methanol yielding blood spiking solution concentrations of 2.27 ng/ml to $3.03 \cdot 10^{3}$ ng/ml HCBD.

HCBD blood standards were prepared by adding an aliquot of the appropriate HCBD blood spiking solution to an accurately weighed sample of approximately 100 mg whole rat blood. After spiking, the blood standard was shaken by hand briefly to ensure mixing. Blood was obtained from Fischer rats via open chest heart puncture using a heparinized syringe.

Extraction method

The HCBD blood standards were extracted with 250 μl of hexane containing the internal standard, TCB. HCBD blood standard concentrations less than 2 ng/ml were extracted with 250 μl of the 2.96 ng/ml TCB stock solution. The 3.26 · 10 ng/ml TCB stock solution was used for extracting HCBD blood standards with concentrations greater than 2 ng/ml. The blood standards were then vigorously vortexted for two min and centrifuged at 600 *g* for one min. Two μl of the organic layer were injected directly onto the GC analytical column. Quantitation of the HCBD blood standards were determined by internal standard calculations.

RESULTS AND DISCUSSION

To determine the percent recovery, blood standards were prepared by adding a known amount of HCBD to 100 mg whole rat blood. All HCBD blood standards were extracted with hexane containing an internal standard, TCB, and analyzed by GC–electron capture detection. Recoveries of HCBD from whole blood ranged from 85.4% to 122.3% and the mean percent recovery for the entire blood concentration range of $1.82 \cdot 10^{-2}$ ng/ml to 6.05 · 10 ng/ml (18.2 ppt–60.5 ppb) was 101.0 ± 7.3% (see Table I). The coefficients of variation for eight HCBD blood concentrations ranged from 0.9% at 3.03 · 10 ng/ml to 11.5% at $1.82 \cdot 10^{-2}$ ng/ml HCBD. Peak height measurements were used for determining percent recovery at $2.50 \cdot 10^{-1}$ ng/ml HCBD and below due to the inability of the CDS-111 to reproducibly identify and quantitate the peak of interest.

No peaks which would interfere with the quantitative analysis of HCBD were present in blank hexane or the internal standard solutions. Blank blood standards

TABLE I

RECOVERY OF HCBD FROM WHOLE RAT BLOOD

Concentration (ng/ml) HCBD in Blood	*Recovery for replicate samples (%)**			*Mean recovery (%)*	*Standard deviation*	*Coefficient of variation*
	No. 1	*No. 2*	*No. 3*			
$6.05 \cdot 10$	101.6	86.3	90.1	92.7	8.0	8.6
$3.03 \cdot 10$	101.0	102.7	102.2	102.0	0.9	0.9
$1.38 \cdot 10$	122.3	115.8	112.9	117.0	4.8	4.1
2.75	104.3	95.2	104.3	101.3	5.3	5.2
1.25	98.5	92.2	97.8	96.2	3.5	3.6
$2.50 \cdot 10^{-1}$**	109.3	100.5	99.0	102.9	5.6	5.4
$4.55 \cdot 10^{-2}$**	99.9	99.9	97.1	99.0	1.6	1.6
$1.82 \cdot 10^{-2}$**	107.8	98.2	85.4	97.1	11.2	11.5
			Average	101.0 ± 7.3***	6.0§	6.1§§

* Each point is the mean of 2 determinations minimum.
** Percent recovery based on peak height measurements.
*** Standard error of mean.
§ Average standard deviation $(x) = (E(x)^2/n)^{1/2}$.
§§ Average coefficient of variation $(y) = (E(y)^2/n)^{1/2}$.

exhibited no background peaks which would interfere with the analysis of HCBD or TCB in whole rat blood. Representative chromatograms of a blank blood standard and an HCBD blood standard are presented in Fig. 1. The retention times of HCBD and TCB were 2.0 and 6.2 min, respectively.

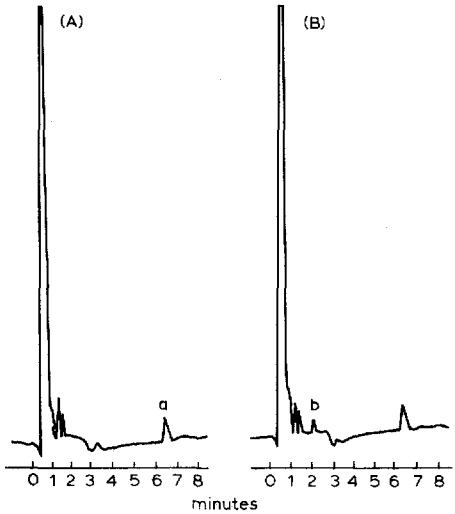

Fig. 1. Representative chromatograms of HCBD in whole rat blood. (A) Chromatogram corresponding to analysis of blank whole rat blood standard containing TCB. (B) Chromatogram corresponding to analysis of HCBD whole rat blood standard containing TCB. Peaks: a = 2.96 ng/ml TCB (× 32 attn.); b = $1.82 \cdot 10^{-2}$ ng/ml HCBD (×4 attn.).

The detector response for both HCBD and TCB was linear for a concentration range of $1.70 \cdot 10^{-2}$ ng/ml to $1.67 \cdot 10^{2}$ ng/ml and $9.17 \cdot 10^{-1}$ ng/ml to $5.55 \cdot 10$ ng/ml, respectively. Linearity was indicated by the linear regression correlation coefficients of 0.9805 and 0.9929 for HCBD and TCB, respectively. HCBD area response linearity below 1.0 ng/ml was determined by proportioning peak height response for a standard below 1.0 ng/ml to a standard above 1.0 ng/ml whose peak height and area were known.

CONCLUSION

The method described in this paper is simple and sensitive for the quantitative determination of HCBD in 100 mg of whole rat blood at concentrations as low as 18 ppt ($1.82 \cdot 10^{-2}$ ng/ml) HCBD.

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