GLC Determination of Free Triclocarban in Blood

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Abstract ◻ A method is presented for the quantitative determination of free triclocarban in rat or human blood. The procedure involves the extraction from blood with acetone, a TLC cleanup, derivatization with *N,O*-bis(trimethylsilyl)acetamide, and GLC using an electron-capture detector. GLC–mass spectral analysis confirmed that the structure of the derivative was a bis(trimethylsilyl) molecule with one group on a nitrogen and the second group attached to the enol tautomer. The method is sensitive to 25 ng (12.5 μg/liter of blood). Recoveries of added triclocarban in the 12.5–50-μg/liter range were between 80 and 90%.

Keyphrases ◻ Triclocarban—GLC analysis, rat and human blood ◻ GLC—analysis, triclocarban, rat and human blood ◻ Disinfectants—triclocarban, GLC analysis, rat and human blood

The bacteriostatic agent triclocarban[1] is used exclusively in bar soaps. It is effective against both Gram-positive bacteria (*e.g.*, staphylococci) and fungi (1, 2). Its chemistry and antimicrobial properties were described previously (3). At the present time, the only methods for the determination of triclocarban are based on UV absorption (4) or HPLC[2]. Neither of these methods approaches the 10-μg/liter sensitivity of the GLC methods developed for the study of hexachlorophene (5) and triclosan[3] absorption.

A direct GLC measurement of triclocarban (4) was unsuccessful because of its low volatility. A method is now presented for the quantitative determination of free triclocarban in human and rat blood following derivatization. The procedure involves extraction from blood with acetone, a cleanup procedure by TLC, conversion to a trimethylsilyl derivative, and analysis by GLC using an electron-capture detector.

EXPERIMENTAL

Reagents—The following were used: triclocarban (>99% purity), acetone (analar grade), toluene (analar), ether (analar), acetonitrile (analar), and *N,O*-bis(trimethylsilyl)acetamide[4] (vacuum distilled).

Apparatus—GLC[5] was conducted on a glass column (1.5 m × 4 mm i.d.) containing 1.5% phenyl methyl silicone[6] and 1.95% trifluoropropyl methyl silicone[7] on 100–120-mesh Supelcon AW-DMCS[8].

All newly prepared columns were conditioned at 240° for 16 hr and then deactivated with 5 μl of hexamethyldisilazane. During analysis, the column and the detector were maintained at 210 and 300°, respectively. Nitrogen was used both as the carrier gas at 75 ml/min and as the purge gas to the ^{63}Ni-electron-capture detector at 25 ml/min.

GLC–Mass Spectrometry[9]—Combined GLC–mass spectrometry of the triclocarban derivative was conducted on a glass column (1.5 m × 4 mm i.d.) containing 3% methyl silicone[10] (OV-1) on 100–120-mesh Diatomite CLQ.

TLC—TLC was conducted on 250-μm silica gel GF plates[11] which had been prewashed overnight in a developing solvent [ether–toluene (1:4)]. After development, the separated bands were visualized by irradiation with a short wavelength (254 nm) UV lamp.

Calculation—The peak height for the trimethylsilyl derivative was measured and compared to a calibration curve prepared each day from known concentrations of triclocarban.

Assay Procedure—A 2.0-ml aliquot of whole blood (stored with edetate dipotassium dihydrate as an anticoagulant) was extracted twice by shaking with 10 ml of acetone. The mixture was centrifuged for 5 min at 1200×*g* after each extraction, and the solvent layers were removed. The combined extracts were placed in a warm water bath and concentrated to approximately 0.2 ml by a nitrogen stream. The concentrated extract was then applied to a silica gel GF plate as a narrow band. At least three aliquots of a standard solution containing 1 μg of triclocarban were spotted onto the plate, and the plate was developed for 10 cm and then allowed to dry at ambient temperature.

A 2-cm wide band of silica gel between the standards, located by irradiation with UV light, was scraped into a 10-ml tube and twice extracted with 10 ml of acetonitrile. The solvent was separated by centrifugation for 5 min at 1200×*g*, and the extracts were combined. The solution was placed on the water bath and concentrated to approximately 5 ml with nitrogen. This solution was transferred to a 10-ml tapered tube and blown to dryness. A 50-μl aliquot of *N,O*-bis(trimethylsilyl)acetamide was added to the residue, the tube was stoppered and placed in a water bath at 60° for 10 min, and a 5-μl aliquot was injected into the chromatograph.

RESULTS AND DISCUSSION

The structure of triclocarban suggests that the germicide might not be particularly well suited to direct GLC analysis. This compound was not eluted from nonpolar stationary phases even at high temperatures. Silylation gave a derivative that could be chromatographed at 210° on a mixed silicone column. Under these conditions, the derivative had a retention time of 7.8 min (Fig. 1).

Elucidation of Derivative Structure—The structure of the derivative was characterized by combined GLC–mass spectrometry. The mass spectrum (Fig. 2) corresponding to the peak from the flame-ionization detector gave significant fragments at *m/e* 458/460/462 (M, molecular ion), 386/388/390 (M − 72, loss of C_3H_8Si), 296/298/300 (further loss of C_3H_9Si + OH), and 73 (C_3H_9Si ion). This pattern confirms that the

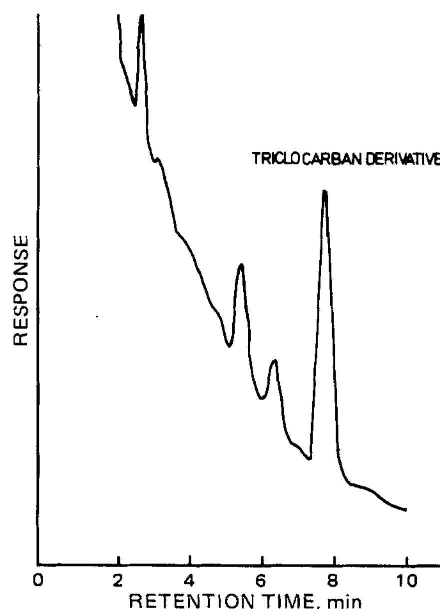

Figure 1—*Chromatogram of extract of whole blood containing 25 μg of triclocarban/liter.*

[1] TCC, 3,4,4′-trichlorocarbanilide, Monsanto.
[2] Paper presented by M. B. Graber and M. M. Goldstein, American Oil Chemists Society, Annual Spring Meeting, Mexico City, Mexico, 1974.
[3] Paper presented by D. R. Hoar and D. J. Sissons, Symposium at the University of Surrey, Surrey, England, September 1975. Triclosan is the generic name for 2′,4,4′-trichloro-2-hydroxydiphenyl ether (Irgasan DP300), Ciba-Geigy.
[4] Pierce Chemical Co.
[5] Pye series 104 chromatograph.
[6] SP 2250.
[7] SP 2401.
[8] Supelco Inc., Bellefonte, Pa.
[9] MS 902 mass spectrometer, AEI.
[10] Perkin-Elmer.
[11] Anachem.

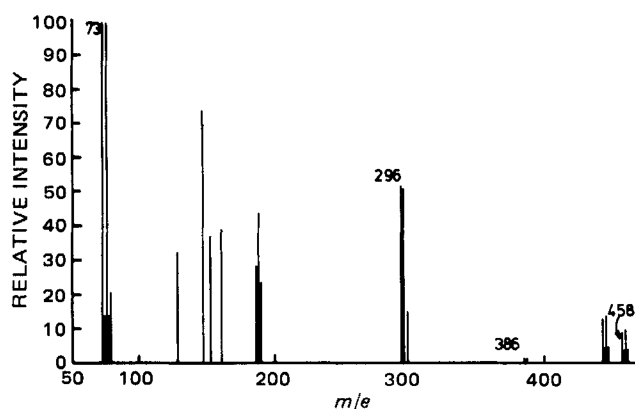

Figure 2—*Mass spectrum* (m/e 50–500) *of the bis(trimethylsilyl) derivative of triclocarban.*

compound was a bis(trimethylsilyl) derivative. The relevant ions each displayed the characteristic trichloro isotope cluster. These particular ions show that only one nitrogen has a trimethylsilyl group while the second trimethylsilyl group is on the enol tautomer.

Examination of the spectra taken at intervals as the peak eluted showed that the ratio of *m/e* 458 to 386 decreased. This finding suggested that the derivative was capable of breaking down to the mono(trimethylsilyl) compound.

It was reported (6) that the drug rafoxanide[12] forms a similar bis(trimethylsilyl) derivative on a phenolic group and a secondary amide group. This derivative was shown to react with active sites on a chromatographic packing, exchanging one of its trimethylsilyl groups for a hydrogen atom to form a mono derivative. This effect was very small when carefully deactivated columns were used. Since electron-capture GLC of standard solutions gave only one peak, the column used for the GLC–mass spectral analysis may not have been completely deactivated.

This phenomenon of silyl donation was not completely unexpected since the amide structure of the derivative is similar to that of the silylating reagent. Therefore, it is important to maintain excess reagent to obtain a quantitative conversion to the bis(trimethylsilyl) derivative and to ensure that the column is completely deactivated. The optimum conditions of derivatization were determined to be 10 min and 60° with *N,O*-bis(trimethylsilyl)acetamide acting as the solvent as well as the derivatizing agent. A linear response was obtained over the range of 5–100 ng of triclocarban.

TLC of Blood Extracts—Ether–toluene (1:4), in which the R_f of triclocarban was 0.4, was by far the most effective solvent system. It separated triclocarban from interfering bands in the blood extract which were concentrated at the origin or solvent front.

Various solvents were evaluated for their ability to extract triclocarban from silica gel TLC plates. It was reported previously (7) that it is frequently necessary to use a solvent with a much greater polarity than the developing solvent. This suggestion was only partly relevant since, although acetonitrile gave 90% recovery, the more polar methanol gave only 41% recovery.

Recovery Experiments—A duplicate series of aliquots containing 25, 50, and 100 ng of triclocarban was blown to dryness in 10-ml tubes. Aliquots (2.0 ml) of whole blood were added to one series of tubes, and the samples were shaken and then analyzed by the described procedure. The second series of standards was taken up in 0.2 ml of acetone and put through the TLC cleanup procedure and the derivatization. The recoveries of these samples relative to a series of standard solutions that had only been concentrated and derivatized are given in Table I.

Table I—**Recovery of Free Triclocarban Added to Whole Blood**

Amount Added to Blood, ng	Amount through Cleanup, ng	Mean Recovery, %	*SD*
100	—	82	±4
—	100	80	±4
50	—	82	±6
—	50	86	±5
25	—	89	±15
—	25	90	±0

The results in Table I show that the recoveries of free triclocarban from whole blood in the 12.5–50-μg/liter range were between 80 and 90%. The results from the standard solutions that had been through only the cleanup procedure and derivatization were in good agreement with the recoveries from the blood samples. These results showed that the losses were occurring during the elution from the TLC plate rather than during the extraction from blood. This finding was confirmed by spotting aliquots containing 50 ng of triclocarban onto a TLC plate, allowing the spots to dry, scraping off the silica gel, and eluting with acetonitrile without developing the plate. The mean recovery was 90%.

Since the sensitivity of this method is comparable to methods used for other germicides, it should be applicable to studies on the absorption and metabolism of triclocarban. The metabolic pathway may be hydroxylation followed by conjugation with glucuronic acid or sulfate (8). Therefore, it is possible that this derivatization method may be applicable to the determination both of the amount and nature of the metabolite(s).

REFERENCES

(1) W. A. Hamilton, *J. Gen. Microbiol.*, **50,** 441 (1968).

(2) T. F. McNamara and M. Steinbach, *J. Am. Oil. Chem. Soc.*, **44,** 478 (1967).

(3) D. P. Roman, E. H. Barnett, and R. J. Balske, *Proc. Sci. Sect. Toilet Goods Assoc.*, **28,** 1213 (1957).

(4) H. S. C. Hong, R. J. Steltenkamp, and N. L. Smith, *J. Pharm. Sci.*, **64,** 860 (1975).

(5) D. G. Ferry and E. G. McQueen, *J. Chromatogr.*, **76,** 233 (1973).

(6) C. P. Talley, N. R. Trenner, G. V. Downing, and W. J. A. VandenHeuvel, *Anal. Chem.*, **43,** 1379 (1971).

(7) E. Stahl, "Thin Layer Chromatography," 2nd ed., George Allen and Unwin Ltd., London, England, 1969, p. 149.

(8) L. G. Scharpf, Jr., I. D. Hill, and H. I. Maibach, *Arch. Environ. Health*, **30,** 7 (1975).

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[12] Rafoxanide is generic name for 3′-chloro-4′-(*p*-chlorophenoxy)-2-hydroxy-3,5-diiodobenzanilide.