Analysis of Trichlorocarbanilide and Triclosan in Soaps by Reverse Phase High Pressure Liquid Chromatography

E.D. GEORGE, E.J. HILLIER, and **S. KRISHNAN,** Bradford Laboratory, Original Bradford Soap Works, Inc., West Warwick, RI 02893

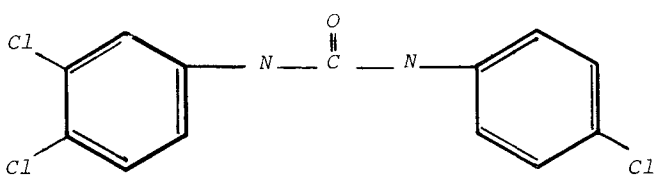
ABSTRACT

A method has been developed for the rapid and direct analysis of bacteriostats (3,4,4'-trichlorocarbanilide and 2,4,4'-trichloro-2'-hydroxydiphenyl ether) in soaps with the aid of reverse phase high pressure liquid chromatography (HPLC) The bacteriostats were conveniently analyzed by UV absorption detection at 280 nm. A typical analysis of bacteriostats by this method requires 15 min for sample preparation and 15 min for the HPLC run. As the system needs no equilibration time (isocratic elution), it is immediately ready for another injection. The method shows excellent accuracy and reproducibility.

INTRODUCTION

Trichlorocarbanilide (TCC) and triclosan, structures labeled I and II respectively, are active ingredients widely used in the manufacture of deodorant soaps. However, up to the present, no quick, convenient, and reliable method has existed for the analysis of these bacteriostats in such soaps.

The methods reported thus far in the literature are primarily based on colorimetry or ultraviolet spectroscopy (1,2). Methods using nuclear magnetic resonance (NMR) high pressure liquid chromatography (HPLC) (4), and gas liquid chromatography for the analysis of degradation products of bacteriostats (5) have also been reported. Analytical methods published by the Ciba-Geigy Corp. (publication no. 2504) mention infrared spectroscopy, potentiometric tritrations, and gas chromatography as feasible means of analyzing triclosan. In general, the published methods suffer from all, or some, of the following drawbacks: (a) time-consuming and elaborate extraction teachniques; (b) the problem of interference by perfume components is not solved to a satisfactory extent or is not addressed at all; and (c) accuracy of about ± 10% (except for the NMR method which is stated to have an accuracy of ± 3%).



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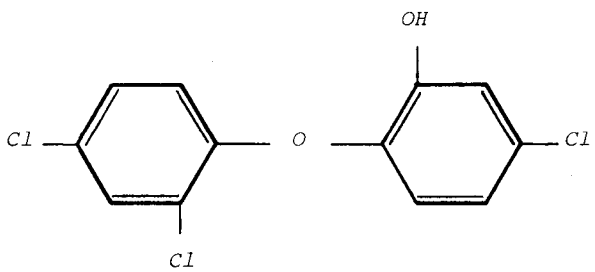

(II)

Authors of previously published papers (5) have indicated difficulties in the analysis of bacteriostats using HPLC. However, with the advent of new separation techniques, it was felt that HPLC could provide an excellent qualitative and quantitative tool for bacteriostat analysis.

The objective of the present study was to develop a methodology for the separation and direct analysis of bacteriostats in commercial deodorant soaps using reverse phase HPLC.

EXPERIMENTAL PROCEDURES

Materials

Samples of TCC and triclosan were obtained through the courtesy of Monsanto Industrial Chemicals Company and Ciba-Geigy Corporation, respectively. The purity of these samples was stated to be greater than 99.5%. Commercial soap base (85:15 sodium tallow:sodium coconut), produced at Original Bradford Soap Works, was used throughout the study. All solvents used were HPLC grade and were filtered through a 0.5μ millipore filter before use. Hydroquinone dimethyl ether was obtained from Eastman Chemicals Products, Inc.

HPLC Apparatus

The apparatus consisted of two constant delivery pumps (Waters Assoc., Milford, MA, Model 6000A) run via a solvent flow programmer (Waters Assoc., Model 660), and fitted with a septumless injector (Waters Assoc., Model U6K) having a 2000$\mu\ell$ loop.

The analytical separation was performed on a Radial Compression Separation System (RCSS) consisting of a Radial Pak A Cartridge (Waters Assoc.) used in conjunction with a Radial Compression Module (Waters Assoc., Model RCM-100). This system was preceded by a guard column containing BONDAPAK Phenyl/CORASIL (Waters Assoc.) of sufficiently low capacity so as not to significantly affect the efficiency of the analytical column. The fixed wavelength ultraviolet absorbance detector (Waters Assoc., Model 440), fitted with a 280 nm wavelength filter, was used to detect and quantitate the bacteriostats.

Sample Preparation

Solutions of varying concentrations (30-300 μg/ml), of both TCC and triclosan, were made by dissolving them in dimethylformamide (DMF).

Stock solutions of 3% soap (containing varying concentrations of TCC and triclosan and 1% fragrance) were prepared by dissolving them in water.

Isolation of the bacteriostats. 1 ml Aliquot of the 3% soap solution was passed through two SEPPAK^{TM} C_{18} cartridges (Waters Assoc.) connected in series. The eluent was discarded.

The cartridges were then washed with a series of solvents (a) 4 ml of water, (b) 2 ml of 40:60 methanol-water, and

(c) 4 ml of water to remove residual soap. All eluents were discarded.

Finally, 4 ml of DMF were passed through the cartridges. This eluent was collected for further analysis.

Hydroquinone dimethyl ether internal standard (.135μg/μl) was mixed 1:1 with the final DMF wash (3 C). This mixture was now ready for injection.

HPLC Operating Conditions

The optimum mobile phase consisted of a 58:42 tetrahydrofuran-water mixture, generated via a solvent flow programmer, during methods development. The flow rate was maintained at 2 ml/min. The sample size for analysis was 30 μl for the bacteriostat standard solutions. The level of bacteriostats present in a given soap was calculated from the standard curves, based on internal standard techniques.

RESULTS AND DISCUSSION

The solubilities of the bacteriostats were experimentally determined in various solvents, and dimethylformamide (DMF) was found to be the most suitable. Standard solutions made with DMF exhibited excellent shelf-life in terms of maintaining solubilities and minimizing evaporation errors.

Sample preparation cartridges were used to isolate the bacteriostats from other constituents (i.e., soap, perfume, color, and other minor additives). Two cartridges connected in series ensured that the soap solutions did not wash the bacteriostats through during the sample preparation since multiple washings of the cartridges with various solvents were needed to remove residual soap and perfume. It was found that a mixture of 40-60 methanol-water removed a considerable amount of fragrance material. When higher concentrations of methanol were used, some of the bacteriostats were removed from the cartridges. The combined washings of water and 40-60 methanol-water eliminated most of the perfume components while effectively isolating the bacteriostats.

The UV spectra of the bacteriostats (Fig. 1) revealed that TCC and triclosan exhibit maximum UV absorbance at 265 nm and 280 nm, respectively; therefore, 280 nm was chosen as a suitable wavelength.

Preliminary investigation was performed on a conventional stainless steel μBONDAPAK C_{18} column (waters Assoc.). Then a mixture of the two bacteriostats was analyzed by HPLC using an isocratic mobile phase, TCC and triclosan coeluted which made it impossible to differentiate the two bacteriostats or determine their concentration.

Baseline separation was achieved for TCC and triclosan using a gradient (curve No. 10) of 60 to 95% THF, at a flow rate of 0.5 ml/min on the μBONDAPAK C_{18} column. This separation was suitable for routine analyses unless the antibacterial soap contained perfume. Perfumed soaps contained components which interfered with the peaks corresponding to the bacteriostats, i.e., they eluted along with the bacteriostats, and gave rise to unresolved peaks (Fig. 2) and erroneous results.

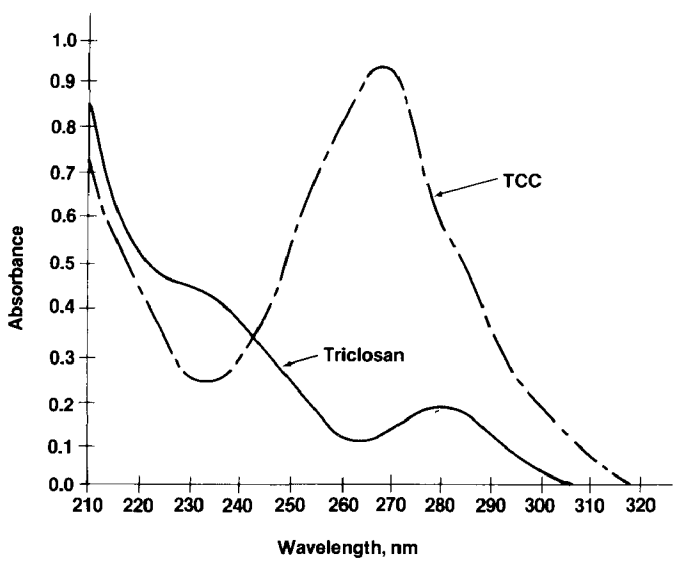

FIG. 1. UV spectra of bacteriostats TCC and triclosan.

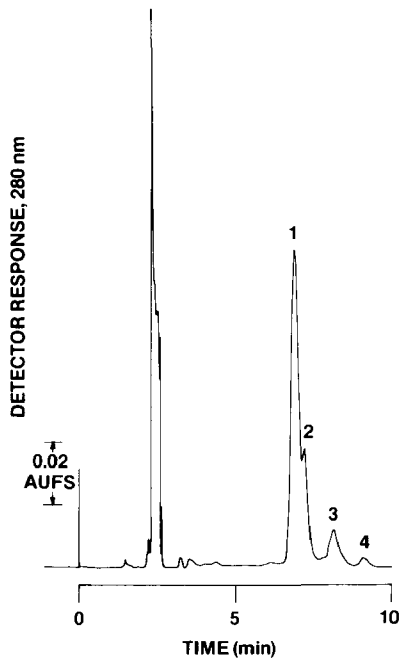

FIG. 2. HPLC chromatogram of bacteriostats and unresolved fragrance components using stainless steel μBondapak C_{18} column. Peak identities: (1) TCC, (2) fragrance component, (3) triclosan, (4) fragrance component.

A technique proposed by Jungermann and Beck (1) involving evaporation as a means of eliminating perfume components was tried. Most of the volatile components were either evaporated or destroyed by this method. However, some high boiling or nonvolatile components still remained after completing this procedure. Unfortunately, these nonvolatile components were the compounds which interfered with the gradient LC analysis of the bacteriostats.

The pH of the mobile phase was modified with additions of acid and base in an attempt to change the elution order of the compounds. However, no significant shifts were observed in the elution patterns. Interferences from the perfume components have also created problems with traditional methods (1).

To circumvent this traditional problem, a more efficient separation system was sought to minimize the interferences by the fragrance components. The conventional stainless steel column was replaced with the Radial Compression Separation System. This separation system increased the system's efficiency and selectivity resulting in excellent resolution of the bacteriostats from the perfume components. The Radial Pak A cartridge provided sufficient resolution to allow the use of an isocratic solvent system consisting of THF:water (58:42) at a flow rate of 2 ml/min

(Fig. 3).

An internal standard was employed for quantitation to minimize operator and sample preparation error. Hydroquinone dimethyl ether was a suitable internal standard since it was stable in the solvent system, did not interfere with any other compound of interest, and exhibited good response at 280 nm (Fig. 4).

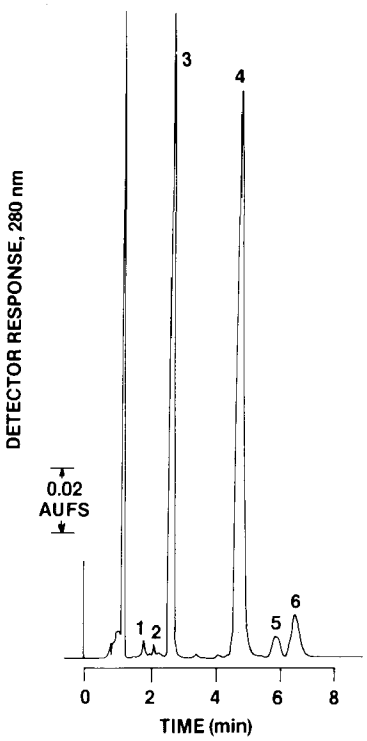

FIG. 3. HPLC chromatogram of bacteriostats using radial compression separation system. Samples were prepared as described in the Experimental section. Peak identities: (1-2) polar fragrance components, (3) hydroquinone dimethyl ether, internal standard, (4) TCC, (5) less polar fragrance components, (6) triclosan.

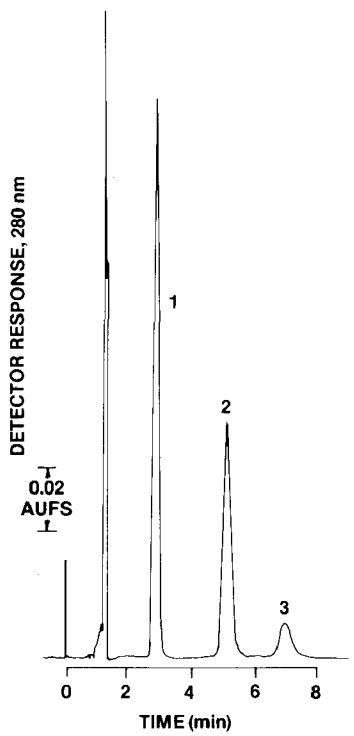

FIG. 4. HPLC chromatogram of a standard mixture containing the bacteriostats and the internal standard. Peak identities: (1) hydroquinone dimethyl ether, (2) TCC, (3) triclosan.

To determine the precision and accuracy of the procedure, calibration curves resulting from injecting various concentrations of the bacteriostats with the internal standard were made. The ratio of sample peak height to internal standard peak height was measured (Table I) and plotted (Fig. 5). [Quantitation by peak height is more accurate than peak area since the peak height is less susceptible to error when measuring overlapping peaks (6)]. Spiked samples were prepared by adding known quantities of bacteriostats to soap. These spiked samples were analyzed by this new HPLC procedure with excellent results as shown in Table II.

Samples of deodorant soap were taken from production runs at Original Bradford Soap Works and analyzed via the new LC procedure, again with good results.

After establishing the calibration curves, the method is applicable to routine analyses since only 15 min is required for sample preparation and 15 min for the LC analysis. The short sample preparation, combined with the precision and accuracy of the LC analysis, provide an analytical method for quality control of these bacteriostats. Although not discussed in this paper, this method would be applicable to

TABLE I

Calibration Table of Standard Mixtures

	Sample no.	Amount injected (μg)	Peak height ratio
TCC	1	0.780	1.058
	2	0.585	0.778
	3	0.390	0.517
	4	0.195	0.257
	5	0.098	0.120
Triclosan	1	0.420	0.1000
	2	0.315	0.0763
	3	0.210	0.0510
	4	0.105	0.0267
	5	0.053	0.0140

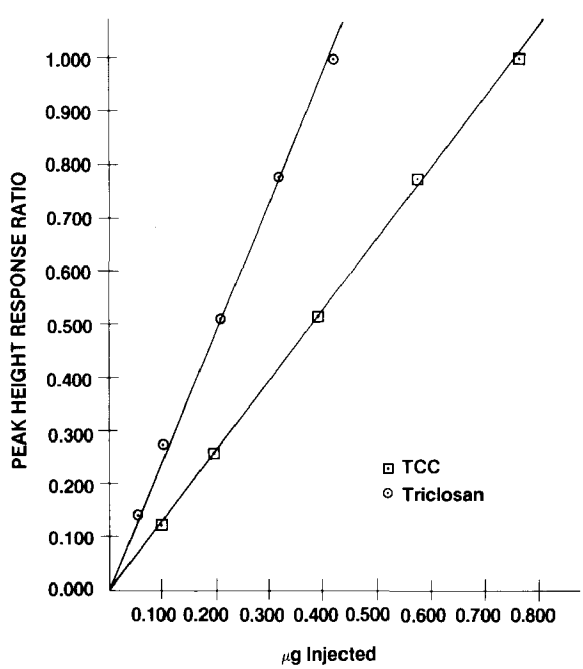

FIG. 5. Calibration curve of bacteriostats and internal standard. Peak height response ratio for triclosan x 10^{-2}.

TABLE II

HPLC Analysis of Standard Mixtures[a]

	Sample no.	Amount added (ng/μl)	Amount recovered (ng/μl)	% Recovery
TCC	1	9.375	9.572	102.1
			9.572	102.1
	2	18.750	18.600	99.2
			18.394	98.1
	3	13.125	12.994	99.0
			12.666	96.5
	4	26.250	26.591	101.3
			26.408	100.6
	5	28.400	27.490	96.8
			28.000	98.6
Triclosan	1	3.750	3.600	96.0
			3.825	102.0
	2	7.500	7.200	96.0
			7.403	98.7
	3	5.625	5.648	100.4
			5.721	101.7
	4	11.250	11.103	98.7
			10.868	96.6
	5	10.400	10.795	103.8
			10.650	102.4

[a]Duplicate injections of each sample.

other bacteriostats in soaps on which this laboratory will report at another time.

ACKNOWLEDGEMENTS

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