



# Disinfectant Analysis

**Phenolic disinfectants can be analyzed rapidly, accurately by a gas chromatographic technique evolved by authors. Data from this procedure compare favorably with those from IR analysis.**

**A**N important phase in the development of Specification MIL-D-51061, Disinfectant, Germicidal and Fungicidal, Phenolic, Dry-Type, was the provision of a suitable analytical method for incorporation in the specification. This material was developed for the disinfection of latrine buckets and as a general purpose housekeeping disinfectant (1). It has a phenol coefficient of 71 and the following composition:

Ingredient	% by weight	
	Minimum	Maximum
Sodium orthophenylphenolate	20.0	—
Sodium 4-chloro-2-phenylphenolate	40.0	—
Sodium 6-chloro-2-phenylphenolate	13.0	17.5
Moisture	14.0	18.0

The above limits of concentration were established from the bactericidal activity of the mixture, and an accurate analysis is therefore important to assure formulation within the limits found to give high performance.

Because of the structural similarity of the three components and their similar chemical behavior, no satisfactory chemical method was available for analysis of all three in combination. Analysis by infrared spectrophotometry was found to be suitable. However, mutual interference at most of the useful wavelengths precluded a simple baseline technique of measurement. It was necessary to analyze the mixture as a three component system by standardiza-

tion of each component at three wavelengths and the setting up and solution of three simultaneous equations (2).

The infrared procedure was adopted, and has been used for several years as the specification method. In practice, additional difficulty was encountered due to lack of additive absorbances of the phenols in the mixture, a prerequisite for accurate analysis of a three component system. A method of correction for absence of additivity was devised (3), but its application was laborious, requiring solution by successive approximation and depending on very precise instrument readings. A less complex and time consuming method was clearly desirable.

The increasing application of gas chromatography to the solution of analytical problems suggested its possible utility for the disinfectant analysis. The gas chromatographic technique is simple, specific and suitable for a wide variety of organic compounds. A procedure which was developed using gas chromatography for the analysis is presented here.

## Column Selection

A solution of orthophenylphenol, 4-chloro-2-phenylphenol

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and 6-chloro-2-phenylphenol in acetone was used for preliminary column scouting. A column temperature of 200°C. or higher was required to elute the chlorophenylphenols. Orthophenylphenol, with a boiling point of 275°C., was first to emerge and was easily separated on all columns. Nonpolar columns such as SE-30 and "Apiezon" L were ineffective in separating the chlorophenylphenols which have boiling points of 312°C. (4-Cl) and 318°C. (6-Cl). On polyphenylether, a column with moderate polarity, some resolution was achieved but not complete separation. Excellent separation was obtained with three highly polar columns, "Carbowax" 20M, diethyleneglycol succinate (DEGS) and XE-60 silicone nitrile. Retention times on the first two columns were excessive and the peaks were very broad. The "Carbowax" at 225°C. and the DEGS at 200°C. required about 45 minutes for analysis. On the XE-60, however, the phenols were eluted in less than 15 minutes at 200°C. and peak shape was much improved. The column was 10 feet x 1/4 inch copper tubing packed with four per cent liquid phase on "Diatoport" S, a "Chromosorb" W support treated with dimethyldichlorosilane to deactivate adsorptive sites on the support. At the four per cent concentration some peak tailing occurred despite the silanizing treatment. The tailing was eliminated by preparation of a second column six feet in length packed with 10 per cent XE-60 on "Diatoport" S.

## Analysis

Operating conditions for the F & M Model 720 gas chromatograph with thermal conductivity detector were chosen: Helium, 30 lbs., 80 ml./min; temperatures, injection port 300°C., column 210°C., detector 250°C.; bridge power, 155 ma; attenuation X4; chart speed, 0.5 in./min; sample size, 10 µl. Order of elution was: o-Phenylphenol 4.8 min.; 6-Cl-2-

phenylphenol 7.4 min.; 4-Cl-2-phenylphenol 11.2 min.

Samples were prepared according to the specification procedure for spectrophotometric analysis (4). A five gram sample of the disinfectant was dissolved in 25 ml. water in a separatory funnel and acidified with 20 ml. 5 N sulfuric acid to convert the sodium salts to the phenol form. The free phenols were extracted three times with 10, 10 and 5 ml. portions of carbon disulfide. The extracts were collected in a 25 ml. volumetric flask, made up to volume with carbon disulfide and dried with sodium sulfate. Ten  $\mu$ l aliquots of this solution were used for chromatography under the operating conditions listed above.

In the first analyses, peak areas were used for calculation, the areas being calculated by multiplying peak height by the peak width at half height. A series of standard solutions of each phenol was chromatographed, and standard curves of peak area vs. concentration were drawn which showed linearity of detector response. The curves could not be used for reading concentrations because of poor day to day reproducibility. It was necessary to run a reference solution with the samples and use the chromatogram of the reference solution for calculation.

Even with careful technique the reproducibility of replicate injections was limited by variation in instrument response and in the amount of sample injected. In order to reduce the variability an internal standard was added to the phenol mixtures. In this procedure, a known amount of standard material is added to the sample and the ratio of the height (or area) of the sample peak to that of the internal standard peak is measured. Height ratios are independent of temperature, flow rate and sample size over limited ranges, and may be used to compensate for minor variations in operating conditions. Parachlorophenol in a concentration of 25 mg./ml. was added to

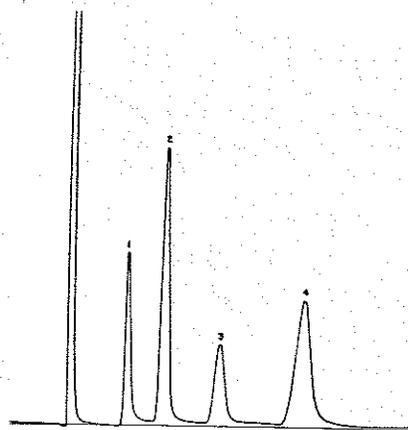


Figure 1. Chromatogram showing separation of (1) p-Chlorophenol; (2) o-Phenylphenol; (3) 6-Cl-2-phenylphenol; and (4) 4-Cl-2-phenylphenol.

all sample and reference solutions. A chromatogram of the phenol mixture with parachlorophenol as internal standard is shown in Figure 1.

Standard curves of height ratio vs. concentration were drawn for the three phenols. (Figure II.) Like the standard curves of peak area vs. concentration they were not reproducible over long periods and it was advisable in each an-

alysis to run a reference mixture containing the internal standard for calculation. A typical reference solution contained the following concentrations in carbon disulfide in a 10 ml. volumetric flask:

	gram
p-Chlorophenol	0.250
o-Phenylphenol	0.500
6-Cl-2-phenylphenol	0.250
4-Cl-2-phenylphenol	0.750

Ten  $\mu$ l aliquots of this solution were chromatographed at the same time as the sample solution.

The data obtained with three phenol mixtures of known concentration were used to evaluate the precision and accuracy of the technique using both height ratios and peak areas for calculation. Table I lists the height ratios and areas obtained from triplicate injections of two of the solutions, and the deviation of each value from the mean of three measurements. The average deviation from the mean was  $\pm 1.1$  per cent for the height ratios and  $\pm 1.6$  per cent

Table I. Precision of Replicate Chromatograms Using Peak Height Ratios and Peak Area Measurement

	Peak height ratio	Deviation from mean %	Peak area cm %	Deviation from mean %
Mixture #1	1.39	+1.5	5.55	-2.3
OPP	1.37	0	5.75	+1.2
39.4 mg/ml	1.35	-1.5	5.75	+1.2
Mean	1.37		5.68	
6-Cl	.526	+1.9	3.21	+1.9
25.3 mg/ml	.519	+0.6	3.16	+0.3
	.504	-2.3	3.07	-2.5
Mean	.516		3.15	
4-Cl	.734	-0.8	6.83	+0.6
73.1 mg/ml	.744	+0.5	6.76	-0.4
	.742	+0.3	6.77	-0.3
Mean	.740		6.79	
Mixture #2	1.56	+2.0	6.58	-2.2
OPP	1.52	-0.7	6.98	+3.7
44.9 mg/ml	1.52	-0.7	6.63	-1.5
Mean	1.53		6.73	
6-Cl	.455	+2.0	2.68	-2.9
22.4 mg/ml	.441	-1.1	2.84	+2.9
	.441	-1.1	2.75	-0.4
Mean	.446		2.76	
4-Cl	.741	+1.2	6.70	-2.6
70.2 mg/ml	.724	-1.1	6.88	0
	.730	-0.3	7.05	+2.5
Mean	.732		6.88	
Average deviation		$\pm 1.1$ %		$\pm 1.6$ %

for the areas. Although the sample size in Table I is small, it may be considered representative. The average deviation of height ratios (areas not measured) for all the standards used in the calibration curves of Figure II was  $\pm 1.0$  per cent. When the data for each component were examined separately, there was some indication that the average deviation for orthophenylphenol was slightly lower than for the other two components. However, no conclusion could be drawn from this limited series.

Concentrations were calculated by treating two of the phenol mixtures as unknown and using the third as a reference for calculation. The recoveries are listed in Table II. Comparison of the recovery errors shows that, in addition to the reduction of variability, the accuracy was improved by the use of height ratios. The maximum relative error in the series was reduced from  $-5.2$  per cent to  $-2.9$  per cent, or from  $-2.1$  per cent to  $-1.2$  per cent absolute error in a sample analysis.

### G.C. vs. I.F. Analysis

Results of analysis of a series of disinfectant samples by gas chromatography and infrared spectrophotometry appear in Table

**Table II. Analysis of Standard Phenol Mixtures.**

Concentrations prepared			Concentrations calculated*					
Component	mg/ml	mg/ml	Height ratios		Peak areas			
			mg/ml	Relative error %	Analysis error %	mg/ml	Relative error %	Analysis error %
Mixture #1	OPP	39.4	40.1	+1.8	+0.4	37.9	-3.8	-0.8
	6-Cl	25.3	26.0	+2.8	+0.4	25.6	+1.2	+0.2
	4-Cl	73.1	71.0	-2.9	-1.2	69.3	-5.2	-2.1
Mixture #2	OPP	50.2	48.8	-2.8	-0.8	51.2	+2.0	+0.6
	6-Cl	25.0	24.6	-1.6	-0.2	25.3	+1.2	+0.2
	4-Cl	75.0	76.9	+2.5	+1.1	78.6	+4.8	+2.0
Mean				$\pm 2.4$	$\pm 0.7$		$\pm 3.0$	$\pm 1.0$

\* Mixture #2 (Table I) used for calculation according to the equation:

$$\text{Conc. mg/ml.} = \frac{\text{HT ratio reference peak}}{\text{HT ratio sample peak}} \times \text{Conc. mg/ml in reference sol'n.}$$

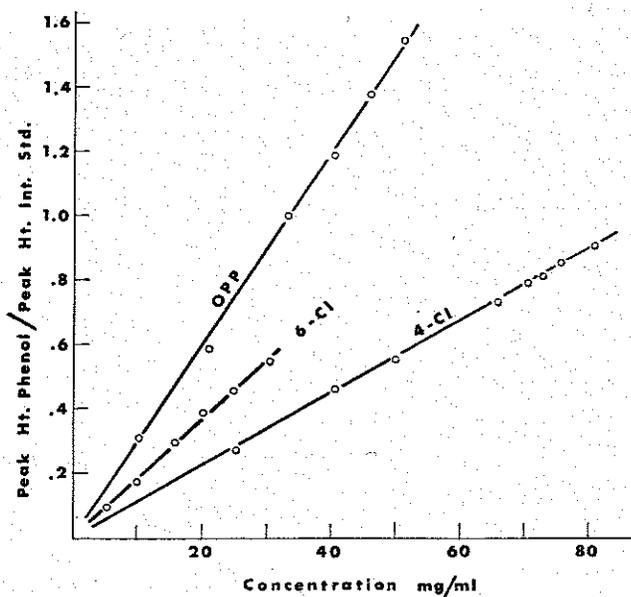
III. While the results are in good agreement, the recovery of all components was lower when measured by gas chromatography. Although the accuracy of analysis of synthetic phenol mixtures may be evaluated by either procedure, corresponding information on the accuracy of sample analysis is not as readily obtained. Formulations of known composition cannot be prepared because the exact purity of the components in the form of the sodium salts is not known. The components are prepared by reacting the phenols with sodium hydroxide, and the reaction product contains water of hydration and a small amount of excess alkali. The

chlorophenylphenols are produced commercially as an isomeric mixture which contains approximately eight per cent orthophenylphenol and three per cent 4, 6-dichloro-2-phenylphenol. The latter, while not measured in the gas chromatographic analysis, may make a contribution to the infrared analysis.

### Interlaboratory Test

The results obtained from sample analysis indicated that the gas chromatographic procedure would be suitable as a specification method to be used for procurement purposes. Before its adoption was proposed as an alternate for

**Figure II. Standard curves for o-phenylphenol; 6-Cl-2-phenylphenol; and 4-Cl-2-phenylphenol. — Peak height ratio vs. concentration.**



**Table III. Analysis of Disinfectant Samples by Gas Chromatography and Infrared Spectrophotometry**

	Components	Weight		
		GC	IR	Difference
Sample #1	OPP (Na salt)	26.4	28.3	1.9
	4-Cl "	39.9	40.8	0.9
	6-Cl "	13.3	13.6	0.3
Sample #2	OPP (Na salt)	26.0	26.9	0.9
	4-Cl "	37.1	39.1	2.0
Sample #3	6-Cl "	13.3	13.6	0.3
	OPP (Na salt)	25.4	27.2	1.8
	4-Cl "	37.2	39.9	2.7
Sample #4	6-Cl "	12.5	13.0	0.5
	OPP (Na salt)	25.8	26.2	0.4
	4-Cl "	37.3	37.8	0.5
	6-Cl "	12.6	13.6	1.0

OPP = Orthophenylphenol  
4-Cl = 4-Chloro-2-phenylphenol  
6-Cl = 6-Chloro-2-phenylphenol

% Sodium orthophenylphenolate =  $\frac{\text{Conc. mg/ml (25) (1.129) (100)}}{\text{sample wt. mg}}$   
% Sodium 6-Cl-2-phenylphenolate and  
% Sodium 4-Cl-2-phenylphenolate =  $\frac{\text{Conc. mg/ml (25) (1.107) (100)}}{\text{sample wt. mg}}$

the infrared method, an arrangement was made to secure data from other sources for an evaluation of its general applicability. For this purpose, a round robin test was conducted in which four laboratories participated, including the Fungicides & Germicides Laboratory at Natick. A pooled sample was prepared from a commercially produced formulation. This material had been exposed in the Arctic for five years and subsequently stored at 0°C. in the laboratory. Its composition was essentially that of the original mixture. One hundred gram quantities of the pooled sample and a copy of the procedure were shipped to each of the participants:

Dow Chemical Co., Midland, Mich.

F & M Scientific Div., Avondale, Pa.

Robert A. Taft Sanitary Engineering Center, Cincinnati.

Results of the round robin test are given in Table IV. The agreement among laboratories was very satisfactory, with an average deviation from the mean for all components of  $\pm 0.5$  per cent, and a maximum deviation of  $-1.0$  per cent.

In addition to the procedure specified for the test, modifications were also used for analysis of the round robin sample. The staff at the F & M laboratories used a flame ionization detector and a carbon disulfide solution of lower concentration with negligible difference in results. Dow investigators performed the analysis without carbon disulfide extraction using a single phase system obtained by direct solution of the disinfectant in water-hydrochloric acid/methanol. The solution was analyzed with a flame detector. Results were higher for each com-

ponent and the total recovery including water was over 100 per cent. When the direct solution procedure was tested in this laboratory, the concentration of 4-chloro-2-phenylphenol was consistently higher by  $1\frac{1}{2}$  to 2 per cent, while the concentrations of the other components were the same as in the extraction procedure. With the XE-60 column there was excessive tailing on the chromatogram from the water peak; a column which does not adsorb water was found to be required for use with the thermal conductivity detector. Because of the convenience and minimal sample manipulation in direct solution, further evaluation will be made of this procedure.

### Discussion

The results of this study show that the gas chromatographic procedure is a suitable and reliable method for disinfectant analysis. It possesses some advantages over the presently used infrared spectrophotometric method in that computations are simpler and less costly instrumentation is required. It is therefore a more practical procedure for routine laboratory use. In the hands of an experienced operator, and in the absence of interfering materials, the precision and accuracy of infrared analysis may equal or exceed that of gas chromatographic analysis. In prac-

tice, however, experience with infrared analysis of the disinfectant has shown that results from different laboratories may be widely divergent. The close agreement among results of the laboratories which participated in the gas chromatographic analysis is in marked contrast to past results. The gas chromatographic procedure is considered to be a very satisfactory analytical method for procurement specification use.

### References

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### Acknowledgement

The authors extend thanks to the following persons for assistance in conducting the round-robin test: Dr. S. A. Shrader and Dr. H. H. Gill of Dow Chemical Company, Dr. Gerald Berg and Mr. Mark McClanahan of Robert A. Taft Sanitary Engineering Center, and to Dr. Gerald Umbreit, Mr. John Wisniewski and Mr. Brian T. Wiley of F & M Scientific Division of Hewlett-Packard.

**Table IV. Gas Chromatographic Analysis of a Pooled Sample of Disinfectant by Four Laboratories.**

Component	Weight %					Average deviation from mean
	Dow	F&M	Natick	Taft	Mean	
OPP (Na Salt)	25.8	26.8	26.3	25.0	26.0	$\pm 0.6$
4-Cl "	36.6	36.6	37.5	37.2	37.0	$\pm 0.4$
6-Cl "	13.3	12.5	13.3	12.5	12.9	$\pm 0.5$