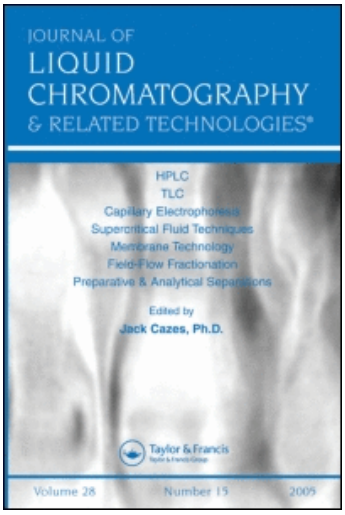

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LIQUID CHROMATOGRAPHIC DETERMINATION OF PHENOLIC COMPOUNDS IN HOSPITAL DISINFECTANT PRODUCTS

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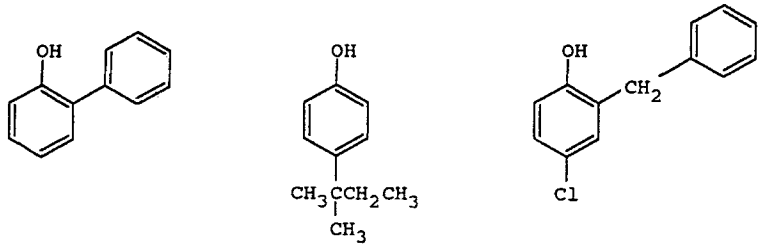
ABSTRACT

A liquid chromatographic method has been developed to quantify phenolic compounds in various hospital disinfectant products. Each product contained two of the following three phenols: o-phenylphenol (OPP), p-tert-amylphenol (PTAP), and o-benzyl-p-chlorophenol (OBPCP). An isocratic mobile phase consisting of methanol and phosphate buffer was used in conjunction with a 5 cm column, which contained 3 micron C-18 packing material, to enact the separation of the phenolic compounds from each other and from product matrix components. Calibration curve data were generated for each phenolic compound to demonstrate linear response over the concentration range of interest. Spiked sample recovery studies were performed to assess the accuracy of the method for the analysis of the phenolic compounds in various product matrices. Finally, photodiode array generated spectra were used to demonstrate the homogeneity of the phenolic compound peaks.

INTRODUCTION

Phenolic compounds are commonly used as the antimicrobial ingredient in hard surface disinfectants which are used in hospitals for general disinfection of noncritical and semicritical areas. The three phenolic compounds (Figure 1) that are used in these types of products are o-phenylphenol (OPP), p-tert-amylphenol (PTAP), and o-benzyl-p-chlorophenol (OBPCP). They exhibit broad-spectrum antimicrobial activity, including gram-negative and gram-positive bacteria. They also exhibit fungicidal, tuberculocidal, and virucidal activity against lipophilic viruses. However, they are not sporicidal and are not used when sterilization is required. These compounds also have a tolerance for organic load and hard water and demonstrate residual activity.(1)

Previous liquid chromatographic studies have involved the analysis of OPP or OPP and OBPCP in conjunction with other phenolic compounds in both normal and reversed-phase chromatographic systems (2 - 7). These studies have determined the amount of OPP in matrices such as urine (2) and immature rat liver microsomal fractions (3). They have also been used to determine the amount of OPP and OBPCP present in cosmetic products when these compounds are used as preservatives (6,7). None of these studies, however, have addressed the liquid chromatographic analysis of OPP, OBPCP, and PTAP in the acidic or alkaline matrices of hospital disinfectant products.



OPP PTAP OBPCP

FIGURE 1. Structures of o-phenylphenol (OPP), p-tert-amylphenol (PTAP), and o-benzyl-p-chlorophenol (OBPCP).

In this study, a fast, simple, high performance liquid chromatography (HPLC) method has been developed which can be used to separate and quantify all three phenolic compounds. The method is applicable for the analysis of the phenolic compounds in three different hospital disinfectant products.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Varian (Walnut Creek, CA, USA) 9095 autosampler with a 50 μL injection loop, a Varian 9010 gradient pump, and a Varian 9065 photodiode array detector. The data were collected and analyzed with a Varian Star workstation. The HPLC column

used was a YMC (Wilmington, NC, USA) ODS-AQ (5 cm x 4 mm I.D., 3 μm packing) with a 0.5 μm precolumn filter (Upchurch Scientific, Oak Harbor, WA, USA).

Reagents

The mobile phase was prepared with HPLC grade reagents only. Fisher (Pittsburgh, PA, USA) water, methanol, potassium phosphate monobasic dihydrate, and 85% o-phosphoric acid were used. OPP (99+% purity, Aldrich, Milwaukee, WI, USA), PTAP (99+% purity, Aldrich), and OBPCP (95+% purity, ICN, Cleveland, OH, USA, recrystallized to a purity of 99+%) were used to prepare standards and spiked samples. The disinfectant products were produced by Calgon Vestal Laboratories (St. Louis, MO, USA). These products will be identified in this paper as Disinfectant 1 (an acidic product which contains OPP and PTAP), Disinfectant 2 (an alkaline product which contains OPP and OBPCP), and Disinfectant 3, (an alkaline product which contains OPP and PTAP).

Mobile Phase Preparation

The isocratic mobile phase consisted of 55% methanol and 45% phosphate buffer (v/v). The phosphate buffer was prepared by dissolving HPLC grade potassium phosphate monobasic dihydrate in HPLC grade water to produce a 50 mM phosphate solution. The pH was then adjusted to 3.0 by the addition of 85% o-phosphoric acid. The mobile phase was

prepared by combining 55% methanol and 45% buffer (v/v) and then vacuum filtering the mixture through a 0.45 μm nylon filter.

Standard and Sample Preparation

OPP, OBPCP, and PTAP standards were prepared by dissolving appropriate amounts of each phenolic compound in mobile phase. Placebo formulations of the disinfectant products were not available. Spiked samples for recovery experiments were prepared by adding known amounts of the appropriate phenolic compounds to disinfectant products of known phenolic compound concentrations. These samples were then diluted with mobile phase. All standards and samples were syringe filtered with a 0.45 μm Gelman Acrodisc LC13 PVDF filter before being analyzed by HPLC.

RESULTS AND DISCUSSION

The goal of this study was to develop a single HPLC method that would not only separate the three phenolic compounds, but could also be used for the analysis of these compounds in one acidic disinfectant product and two alkaline disinfectant products. If the same chromatographic conditions could be used for all three products, then this would represent a savings in terms of analysis time since no chromatographic change over would be required when switching the analysis from one product to another. It was

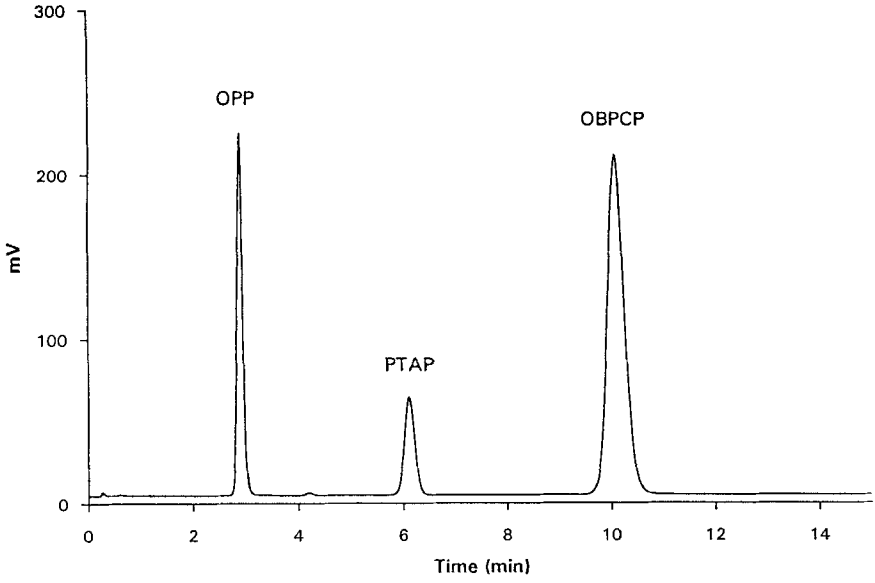


FIGURE 2. Chromatogram of OPP, PTAP, and OBPCP standards.

also important that the final method be isocratic so that it could more easily be transferred to other laboratories. The final chromatographic conditions consisted of an isocratic mobile phase of 55% / 45% (v/v) methanol and 50 mM phosphate buffer at pH 3.0, respectively, a flow rate of 1.5 mL/min, an injection volume of 50 μL, UV detection at 220 nm, ambient column temperature, and an analysis time of 15 minutes.

Calibration Curve Data

Calibration curve data were generated for each of the three phenolic compounds. Two runs (analyses) of each phenolic compound standard

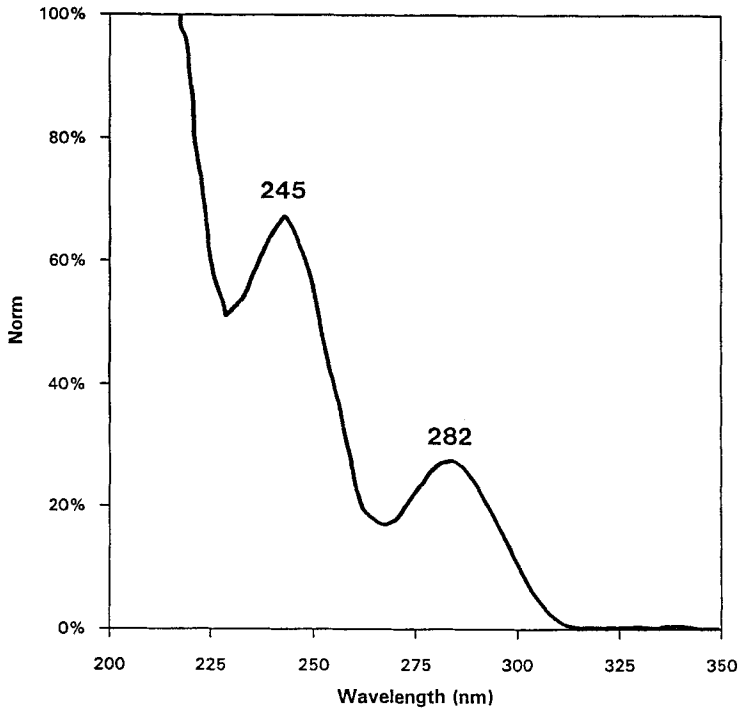


FIGURE 3. Photodiode array detector generated UV spectrum of OPP.

series were performed but no limit of detection or quantitation values were established since this is usually not an issue for this type of product analysis. A typical chromatogram of the standards is found in Figure 2 and the photo diode array generated UV spectra for the phenolic compounds are found in Figures 3, 4, and 5. The Run 1 calibration curves consisted of six OPP standards ranging from 9.85 ppm to 60.7 ppm, six PTAP standards ranging from 11.0 ppm to 59.9 ppm, and five OBPCP standards ranging from 55.0 ppm to 273 ppm. The Run 2 calibration curves consisted

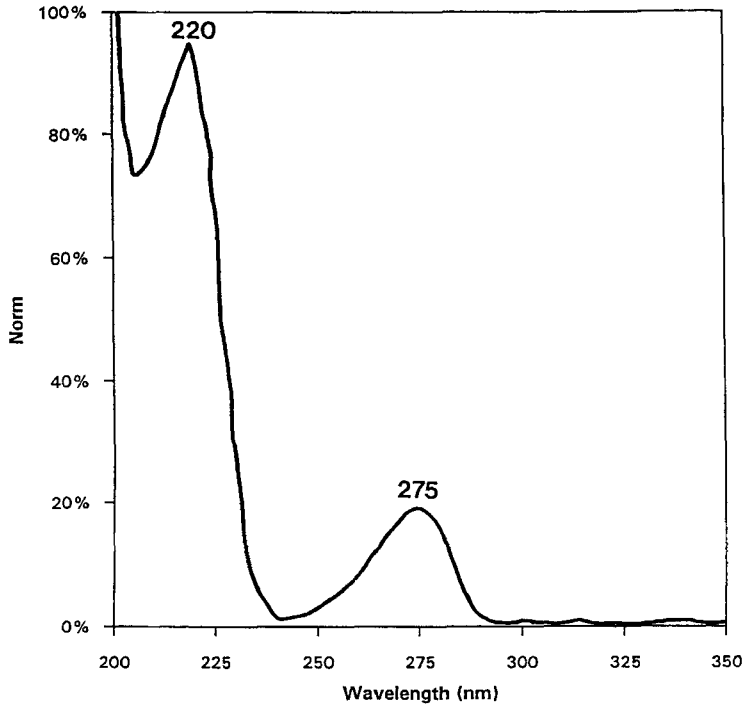


FIGURE 4. Photodiode array detector generated UV spectrum of PTAP.

of six OPP standards ranging from 11.0 ppm to 61.5 ppm, six PTAP standards ranging from 12.0 ppm to 58.4 ppm, and six OBPCP standards ranging from 61.3 ppm to 310 ppm. A linear response was found over each concentration range for all the phenolic compounds. Table 1 contains a summary of the OPP, PTAP, and OBPCP calibration curve data.

Analysis of Formulated Disinfectant Products

In order to assess the accuracy of the assay, spiked samples were prepared for each of the disinfectants and then analyzed for phenolic

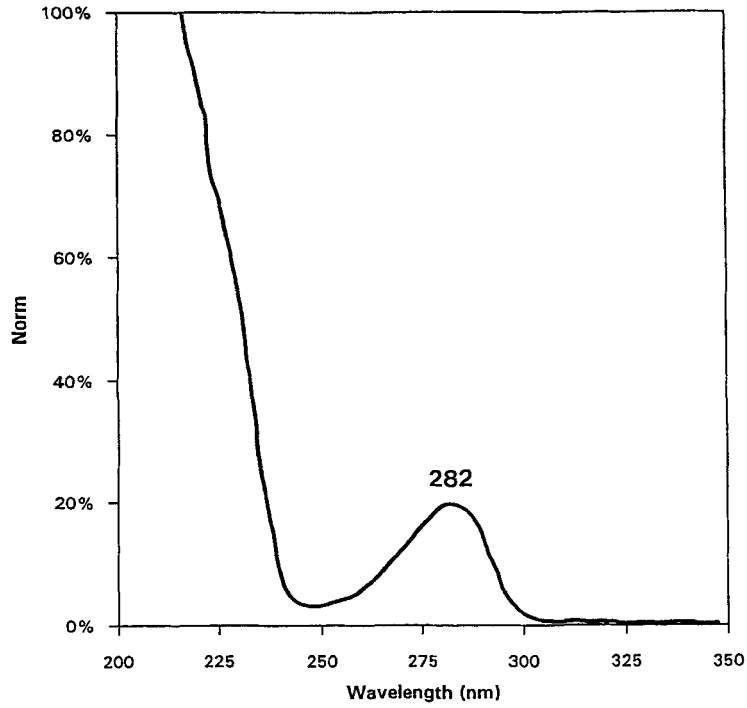


FIGURE 5. Photodiode array detector generated UV spectrum of OBPCP.

TABLE 1. Summary of Calibration Curve Data.

Run	R^2	Slope	y-intercept
OPP-1	0.9999	12555	6551
OPP-2	1.0000	12330	4723
PTAP-1	1.0000	6705	714
PTAP-2	1.0000	6591	-423
OBPCP-1	1.0000	7588	4800
OBPCP-2	1.0000	7419	4695

TABLE 2. Spiked Sample Recovery Data for Disinfectant 1.

Analyte	Theoretical Sample Conc, ppm	Theoretical Spike Conc, ppm	Expt.Total Conc, ppm	Expt. Spike Conc, ppm	% Recovery
OPP	41.93	2.81	44.82	2.89	103
	40.50	5.97	46.52	6.01	101
	40.54	9.05	49.71	9.16	101
	37.78	3.18	40.94	3.16	99.2
	41.43	5.97	47.41	5.98	100
	40.43	9.48	49.85	9.42	99.3
Mean OPP ± Std Dev					100.6 ± 1.4
PTAP	42.06	2.78	44.84	2.77	99.8
	40.64	5.90	46.55	5.92	100
	40.68	8.94	49.73	9.06	101
	37.91	3.14	41.11	3.20	102
	41.56	5.90	47.60	6.03	102
	40.57	9.36	49.95	9.39	100
Mean PTAP ± Std Dev					100.8 ± 1.0

compound content. The spiked sample recovery data are found in Table 2 for Disinfectant 1, in Table 3 for Disinfectant 2, and in Table 4 for Disinfectant 3. Since true placebos were not available, the phenolic compounds were spiked into disinfectant samples that already contained the phenolic compounds. The resultant samples were analyzed in order to generate percent recovery data in the following manner. The "Theoretical

TABLE 3. Spiked Sample Recovery Data for Disinfectant 2.

Analyte	Theoretical Sample Conc, ppm	Theoretical Spike Conc, ppm	Expt.Total Conc, ppm	Expt. Spike Conc, ppm	% Recovery
OPP	23.15	5.52	28.72	5.57	101
	25.55	9.54	35.12	9.57	100
	27.59	3.00	30.61	3.02	101
	25.45	5.54	31.05	5.60	101
	29.24	9.35	38.57	9.32	100
Mean OPP ± Std Dev					101 ± 0.5
OBPCP	118.17	27.81	145.73	27.56	99.1
	130.40	48.05	178.40	48.01	100
	140.81	15.12	156.08	15.26	101
	129.88	27.91	157.97	28.09	101
	149.26	47.09	195.92	46.66	101
Mean OBPCP ± Std Dev					100 ± 0.9

Sample Concentration" value was determined by analyzing the disinfectant for its phenolic compound content. The "Theoretical Spike Concentration" value was determined based upon the amount of the phenolic compound that was added to the disinfectant. The "Experimental Total Concentration" value was determined by analyzing the sample after it had been spiked with the appropriate phenolic compounds. The "Experimental Spike Concentration" value was determined by subtracting the "Theoretical

TABLE 4. Spiked Sample Recovery Data for Disinfectant 3.

Analyte	Theoretical Sample Conc, ppm	Theoretical Spike Conc, ppm	Expt.Total Conc, ppm	Expt. Spike Conc, ppm	% Recovery
OPP	45.27	3.01	48.36	3.09	103
	46.19	5.92	52.13	5.94	100
	48.08	9.66	57.77	9.69	100
	46.58	2.98	49.63	3.04	102
	48.92	5.70	54.57	5.64	98.9
	49.95	9.05	58.93	8.98	99.2
Mean OPP ± Std Dev					101 ± 1.6
PTAP	39.88	2.96	42.89	3.01	102
	40.69	5.81	46.57	5.88	101
	42.36	9.49	52.02	9.67	102
	41.04	2.93	44.02	2.99	102
	43.10	5.59	48.68	5.59	100
	44.00	8.89	53.01	9.01	101
Mean PTAP ± Std Dev					101 ± 0.8

Sample Concentration" value from the "Experimental Total Concentration" value. Finally, the % Recovery value was determined by [(Expt. Spike Conc) (100)] ÷ (Theoretical Spike Conc). Typical mean recovery values of 100% or 101% demonstrated accuracy for the quantitation of relevant phenolic compounds in each product.

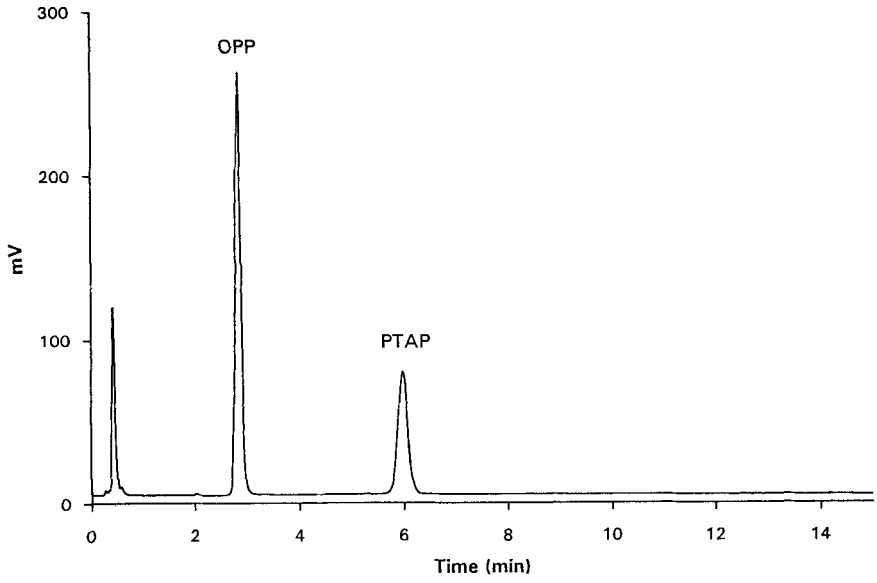


FIGURE 6. Chromatogram of a Disinfectant 1 sample.

The photodiode array detector was used to assess peak homogeneity. Photodiode array generated purity parameter (PuP) data can be used to determine if the individual phenolic compound peaks are homogeneous in each product matrix. For a given wavelength range, the PuP is the average wavelength weighted by the square of the absorbance (8). PuP = $[(\Sigma A_i^2)(\lambda_i)] \div \Sigma A_i^2$, where A_i is absorbance at wavelength λ_i. A series of five spectra were collected across each phenolic compound peak in each disinfectant product matrix in order to assess the homogeneity of each peak. If the peak is homogeneous, then the PuP will remain essentially the same as spectra are collected across the peak. Representative

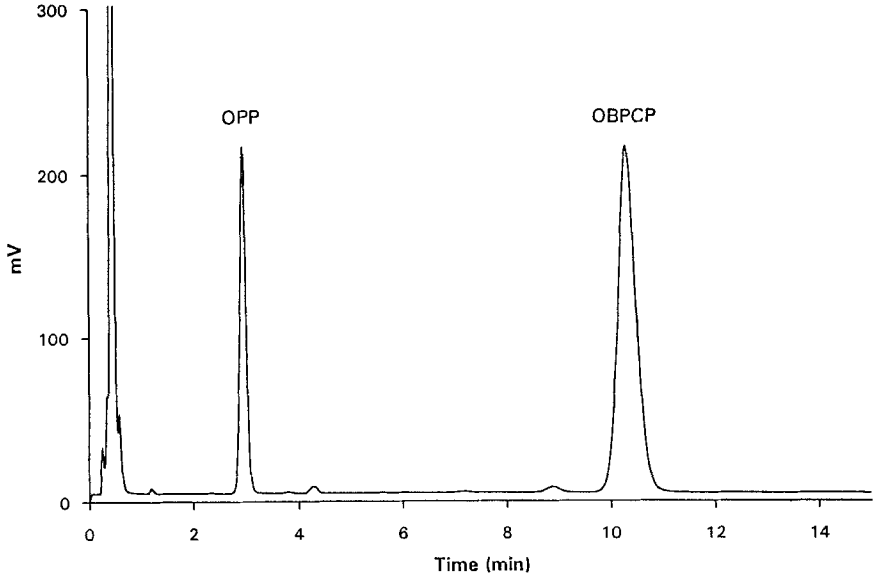


FIGURE 7. Chromatogram of a Disinfectant 2 sample.

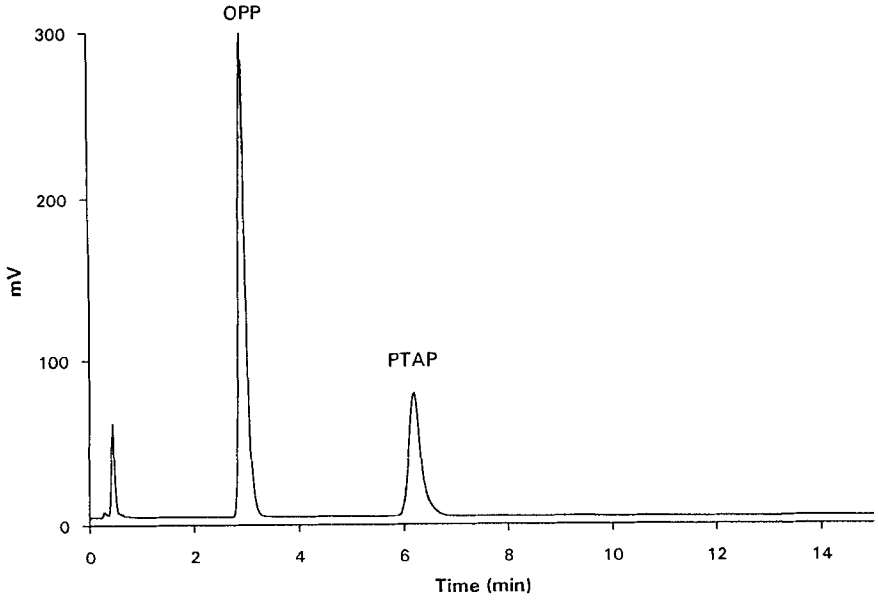


FIGURE 8. Chromatogram of a Disinfectant 3 sample.

TABLE 5. Purity Parameter (PuP) Data for Disinfectant 1.

Spectrum #	OPP PuP (210 to 367 nm)	PTAP PuP (210 to 367 nm)
1	224.34	220.30
2	224.25	220.32
3	224.33	220.32
4	224.17	220.33
5	224.29	220.33
Mean ± Std Dev	224.27 ± 0.07	220.32 ± 0.01

TABLE 6. Purity Parameter (PuP) Data for Disinfectant 2.

Spectrum #	OPP PuP (210 to 367 nm)	OBPCP PuP (210 to 367 nm)
1	224.32	217.60
2	224.14	217.53
3	224.40	217.63
4	224.39	217.61
5	224.07	217.49
Mean ± Std Dev	224.26 ± 0.14	217.57 ± 0.05

TABLE 7. Purity Parameter (PuP) Data for Disinfectant 3.

OPP Spectrum #	OPP PuP (210 to 367 nm)	PTAP PuP (210 to 367 nm)
1	224.04	220.44
2	224.02	220.45
3	223.93	220.41
4	224.02	220.34
5	223.77	220.44
Mean ± Std Dev	223.96 ± 0.11	220.42 ± 0.04

chromatograms for each disinfectant product are found in Figures 6 through 8. The PuP data in Tables 5 though 7 indicate that the individual phenolic compound peaks were homogeneous in each disinfectant product matrix.

CONCLUSION

A simple, fast HPLC method was developed to quantify the phenolic compounds OPP, PTAP, and OBPCP in various hospital disinfectant products. Calibration curve data indicated that the response of each phenolic compound was linear over the concentration ranges of interest. The analysis of spiked recovery samples demonstrated that the method was accurate and photodiode array spectral data indicated that each phenolic compound peak was homogeneous in each disinfectant matrix.

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