


Liquid Chromatography

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Analysis of Chlorophenols in Water by UHPLC with PDA Detection

Introduction

Chlorophenols are common contaminants due to their widespread usage and are introduced into the

environment as a result of chemical and pharmaceutical manufacturing. They can also be found in industrial waste, pesticides and insecticides or as degradation products of complex chlorinated hydrocarbons.¹

Chlorophenols in water have been of particular public concern due to their high toxicity and persistence within the environment.² In drinking water, chlorophenols are present as bi-products from the reaction of chemical agents with phenolic acids during water disinfection and chlorination.³

According to the U.S. Environmental Protection Agency (EPA), the human health ambient water quality criteria for 2-chlorophenol, 3-methyl-4-chlorophenol, 2,4-dichlorophenol, and pentachlorophenol are 20 ppm, 500 ppm, 10 ppb and 1 ppb, respectively. Currently, in the U.S., there is no regulation for 2,4,6-trichlorophenol as it is entirely banned from use.^{4,5,6}

This application will focus on the solid phase extraction (SPE) and HPLC analysis of chlorophenols in three tap water samples. Method conditions and performance data, including linearity and repeatability, are presented.

Experimental

Hardware/Software

For all chromatographic separations, a PerkinElmer Altus™ UPLC® system was used, including the Altus A-30 Solvent/Sample Module, integrated vacuum degasser, A-30M Column Module and A-30 PDA (photodiode array) detector with a 25-mm flow cell. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 Chromatography Data Software (CDS) platform.

Method Parameters

The LC method parameters are shown in Table 1.

Table 1. LC Method Parameters.

Column:	PerkinElmer Brownlee™ SPP 2.7 μm, 3.0 x 100-mm (Part# N9308410)																														
Mobile Phase:	Solvent A: 0.1% acetic acid in water Solvent B: 0.1% acetic acid in methanol (MeOH) Solvent program: <table border="1" data-bbox="352 1146 785 1304"> <thead> <tr> <th></th> <th>Time (min)</th> <th>Flow Rate (mL/min)</th> <th>%A</th> <th>%B</th> <th>Step</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Initial</td> <td>0.8</td> <td>50.0</td> <td>50.0</td> <td></td> </tr> <tr> <td>2</td> <td>4.5</td> <td>0.8</td> <td>35.0</td> <td>65.0</td> <td>6</td> </tr> <tr> <td>3</td> <td>8.0</td> <td>0.8</td> <td>0.0</td> <td>100.0</td> <td>6</td> </tr> <tr> <td>4</td> <td>8.1</td> <td>0.8</td> <td>50.0</td> <td>50.0</td> <td>11</td> </tr> </tbody> </table>		Time (min)	Flow Rate (mL/min)	%A	%B	Step	1	Initial	0.8	50.0	50.0		2	4.5	0.8	35.0	65.0	6	3	8.0	0.8	0.0	100.0	6	4	8.1	0.8	50.0	50.0	11
	Time (min)	Flow Rate (mL/min)	%A	%B	Step																										
1	Initial	0.8	50.0	50.0																											
2	4.5	0.8	35.0	65.0	6																										
3	8.0	0.8	0.0	100.0	6																										
4	8.1	0.8	50.0	50.0	11																										
Analysis Time:	8.0 min.: 3-min. injection delay time between injections																														
Flow Rate:	0.8 mL/min.																														
Pressure:	6700 psi/462 bar (maximum)																														
Oven Temp.:	45 °C																														
Detection:	Altus A-30 PDA; wavelength: 280 nm																														
Injection Volume:	5 μL																														
Sampling (Data) Rate:	5 pts./sec																														

Solvents, Standards and Samples

All solvents and diluents used were HPLC-grade and filtered via 0.45-μm filters.

The standards were obtained from Sigma Aldrich, Inc® (Milwaukee, WI) and consisted of 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), 4-chloro-3-methylphenol (4-Cl,3-MP) and pentachlorophenol (PCP). Individual 500-ppm stock solutions of each analyte were prepared using MeOH as the diluent. A standard mix solution containing 100 ppm of each analyte was prepared by adding equal amounts of each individual stock solution. From this, a 10-ppm working standard was prepared by spiking 4.5 mL of HPLC-grade water with 0.5 mL of standard mix. This working standard was then acidified to pH <2 with HCl.

Tap water samples were collected from Princeton, NJ, Brooklyn, NY, and Shelton, CT. All samples were filtered through 0.45-μm filters and acidified with HCl to pH <2.

For both the working standard and samples, Supra-Poly SPE columns (100 mg/mL; P/N N9306403; Perkinelmer Inc.), were initially conditioned with 6 mL MeOH and then equilibrated with 6 mL acidified water (pH < 2), each drawn through under gentle vacuum. After conditioning, the working standard and the water samples were each passed through one of the pre-conditioned SPE cartridges, using similar vacuum and flow conditions. The cartridges were then dried under gentle vacuum and analytes were subsequently eluted with 2.0 mL of MeOH.

After SPE extraction of the 10-ppm spiked working standard, all lower level working standards were then prepared from this extract via serial dilution in MeOH.

Prior to injection, all calibrants and samples were filtered through 0.45-μm filters to remove small particles.

Results and Discussion

Figure 1 shows the chromatographic separation of the 5-ppm working standard, using the optimized conditions described above. The analysis time was under eight minutes, with all five analytes well resolved.

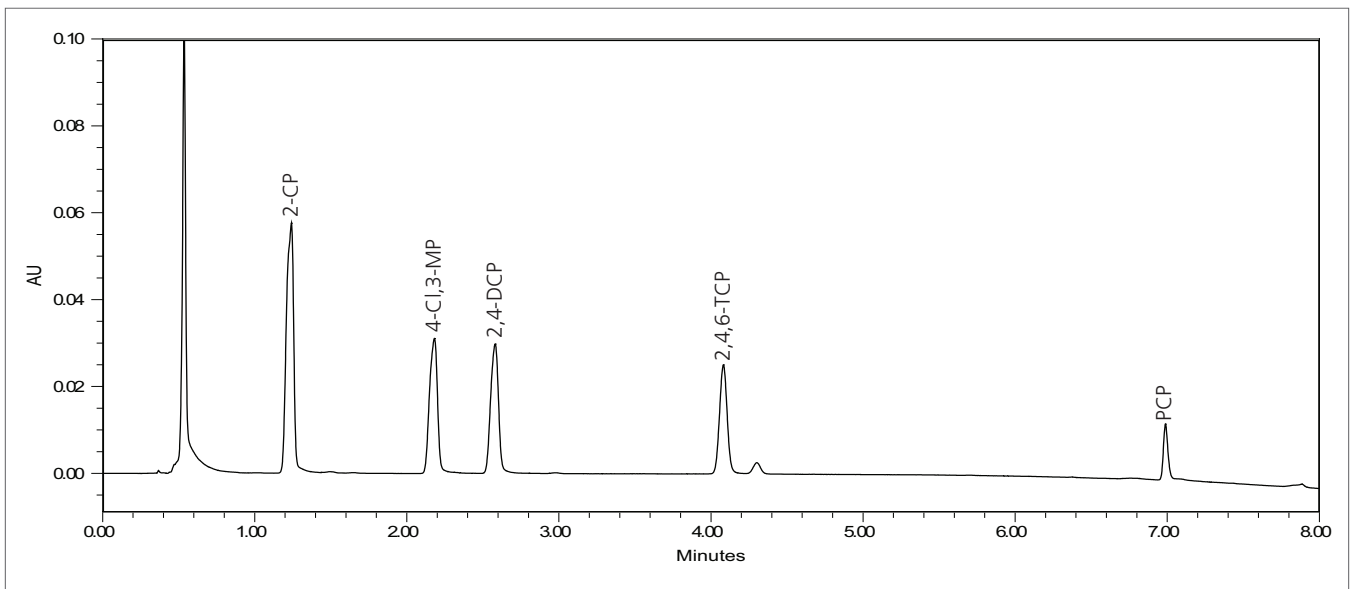


Figure 1. Chromatogram of the 5-ppm working standard.

Figure 2 shows the overlay of 10 replicate 5-ppm working standard injections, demonstrating exceptional reproducibility. The retention time %RSD for PCP was 0.027%.

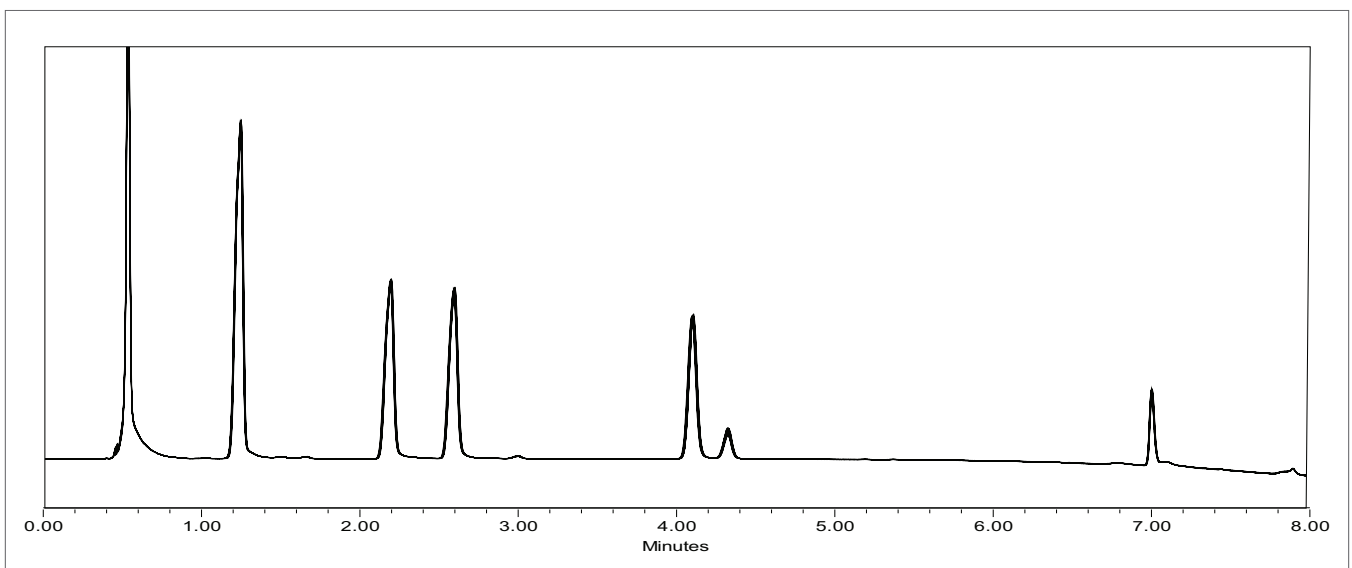


Figure 2. Overlay of 10 replicates of the 5-ppm working standard.

Figure 3 shows the representative calibration results for 2-CP, 4-Cl,3-MP and 2,4,6-TCP over a concentration range of 0.3 to 7.0 ppm. All the standards followed a linear (1st order) fit and had R² coefficients > 0.999 (n = 3 at each level).

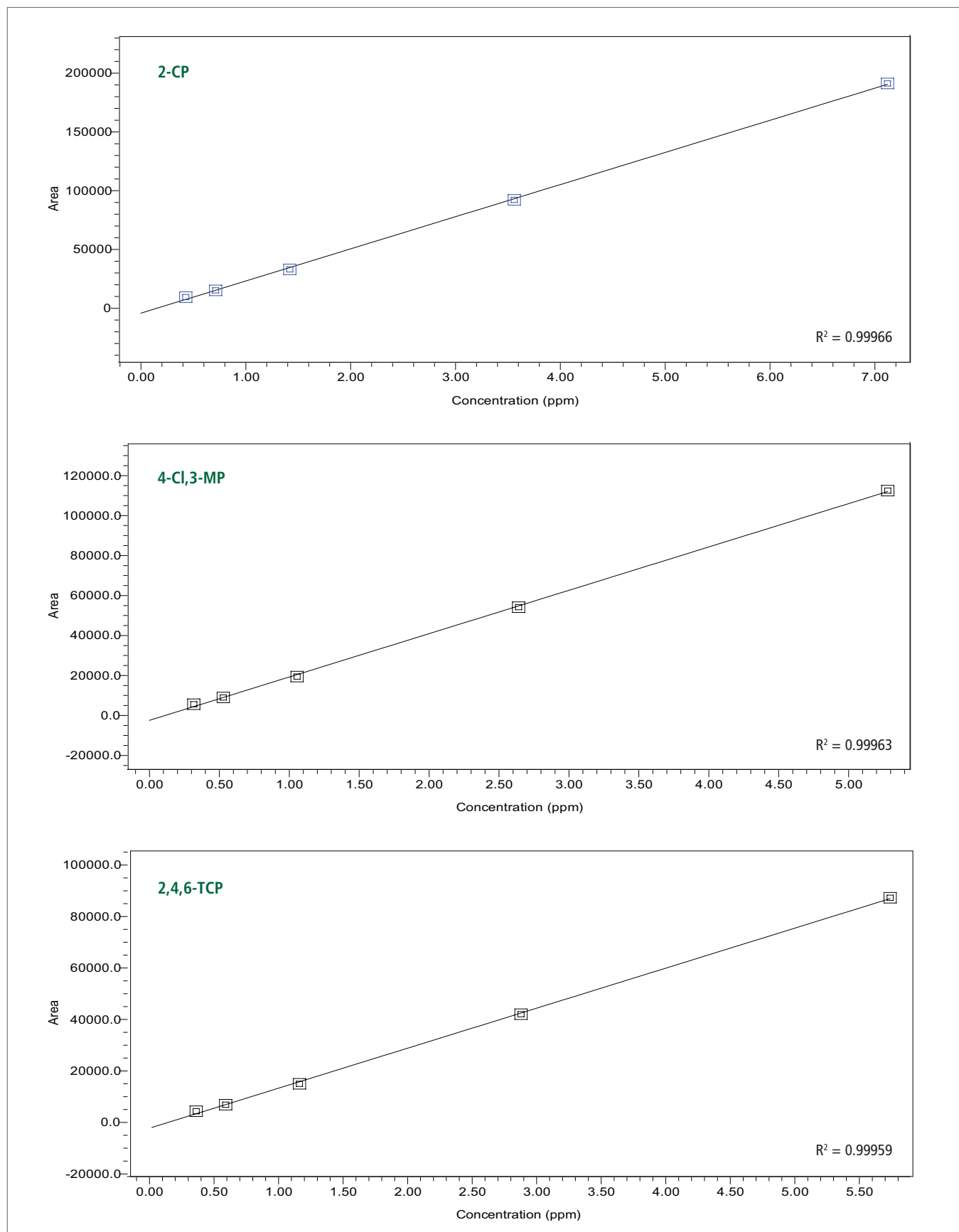


Figure 3. Results of 5-level calibration sets for 2-chlorophenol (2-CP), 4-chloro-3-methylphenol (4-Cl,3-MP), and 2,4,6-trichlorophenol (2,4,6-TCP).

As listed in Table 2, LOD (limit of detection) and LOQ (limit of quantitation) levels were established for each analyte, based upon a signal-to-noise (s/n) of $> 3/1$ for the LOD and $>10/1$ for the LOQ.

Using the same chromatographic conditions, three tap water samples were analyzed (Princeton, NJ; Brooklyn, NY; Shelton, CT) and the results are shown in Figure 4. Comparing the chromatograms of the three tap water samples with the 0.5 ppm working standard, the samples were found to contain no detectable levels of the five chlorophenols. PDA spectral analysis confirmed that the peak eluting in the water samples at approximately 1.1 minutes, just prior to the first analyte of interest, is not associated with 2-CP. It should be

Table 2. LOD and LOQ values for the chlorophenol analytes.

Analyte	LOD (ppb)	LOQ (ppb)
2-CP	3.4	11
2,4-DCP	8.3	28
2,4,6-TCP	13	43
4-Cl,3-MP	6.3	21
PCP	27	89

noted that the 0.5 ppm working standard also served as a spiked water sample, having undergone the same sample preparation routine as the samples. As shown in the chromatogram of the 0.5 ppm working standard, the minor relatively-broad matrix "component" eluting at 7.0 minutes was found to have little effect on the quantitability of the PCP.

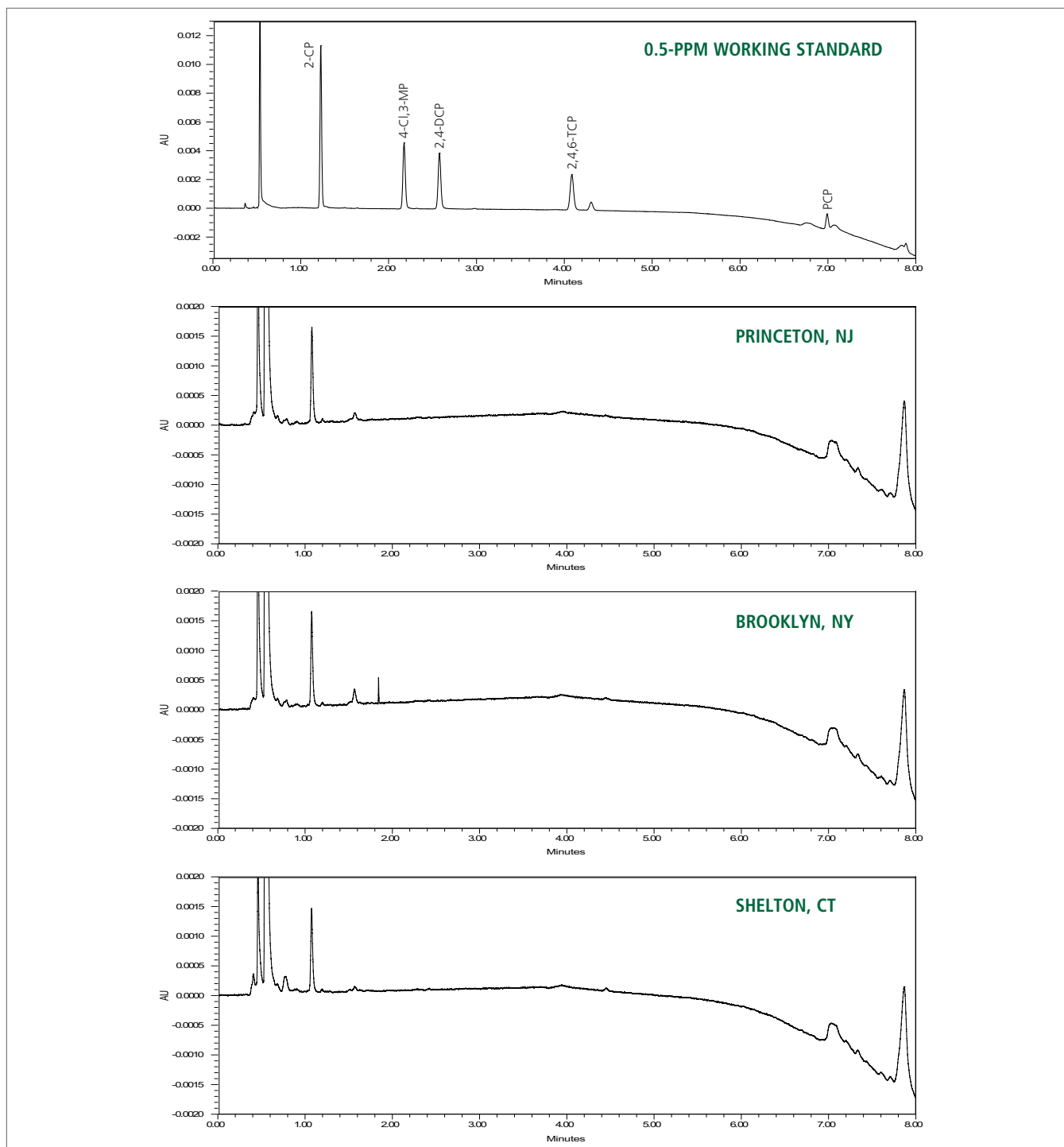


Figure 4. Chromatograms of the water samples and 0.5-ppm working standard.

Conclusion

This work has demonstrated the effective chromatographic separation of 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 4-chloro-3-methylphenol and pentachlorophenol using a PerkinElmer Altus HPLC system with PDA detection. The results exhibited very good retention time repeatability as well as excellent linearity over the tested concentration ranges. The LOD for 2-chlorophenol, 2,4-dichlorophenol and 4-chloro-3-methylphenol were all found to be below their EPA-regulated limits.

This work also focused on the chlorophenol analysis of three tap water samples, comparing them both chromatographically and quantitatively. This is especially relevant in today's environmentally-sensitive climate, and the uncertainties surrounding potential health effects of chlorophenol exposure.

References

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