Simple HPLC–UV Analysis of Phenol and Its Related Compounds in Tap Water after Pre-Column Derivatization with 4-Nitrobenzoyl Chloride

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Abstract

The purpose of this study is to develop an HPLC-UV (280 nm) method for simultaneous determination of phenol, five chlorophenols (2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, and 2,4,6-trichlorophenol), and three phenylphenols (2-phenylphenol, 3-phenylphenol, and 4-phenylphenol) in tap water after pre-column derivatization with 4-nitrobenzoyl chloride. Standard curves were obtained after derivatization with 4-nitrobenzoyl chloride in borate buffer (pH 8.5) at 50°C for 1 min. The nine 4-nitrobenzoyl derivatives were well separated in less than 15 min on a Cholesterol column. Calibration plots were linear in the range of 0.02 ~ 0.12 to 0.9 mg/L, with \( r^2 \) values \( \geq 0.9928 \), for all compounds. The lower limits of detection were 0.006 to 0.05 mg/L. The recovery values from tap water spiked with a standard mixture of test compounds were satisfactory. While the levels of phenol, five chlorophenols, and three phenylphenols in tap water were below the lower limit of determination, our method is expected to be useful for monitoring and/or identifying environmental water samples that are contaminated with these compounds, i.e., for assessing compliance with the official guidelines of the World Health Organization.

Keywords

Phenol, Chlorophenol, Phenylphenol, 4-Nitrobenzoyl Chloride, Derivatization

1. Introduction

Phenol is present as a pollutant in the aquatic environment because of its widespread use for the synthesis of dyes and drugs, and its presence in various
commercial products. Chlorophenols (2-chlorophenol, 2-CP; 4-chlorophenol, 4-CP; 2,4-dichlorophenol, 2,4-DCP; 2,6-dichlorophenol, 2,6-DCP; and 2,4,6-trichlorophenol, 2,4,6-TCP) are malodorous even at very low concentrations in the aquatic environment, and may be present in drinking water as a result of disinfection processes employing chlorination, which result in chlorination at the o- and/or p- position(s) of phenol, if it is present. These compounds may also be formed by the reaction of hypochlorite with phenolic acids and during the degradation of phenoxy herbicides [1]. The World Health Organization (WHO) guideline value for 2,4,6-TCP is 0.2 mg/L, and concentrations of CPs in drinking-water are usually less than 0.001 mg/L [1]. The maximum permissible level of total phenols is 0.5 mg/L in drinking water, and the concentrations of individual phenols must not exceed 0.1 mg/L according to the United States Environmental Protection Agency and the European Union regulations [2] [3] [4]. On the other hand, the maximum permissible level of total phenols is less than 0.005 mg/L in drinking and tap water and less than 5 mg/L in industrial waste water according to the Japanese Water Pollution Control Law.

Since 2-phenylphenol (2-PP) has activities as a disinfectant, bactericide, and virucide, it is used in households, industry, and hospitals to disinfect surfaces and is also utilized as a preservative in cosmetics, plastics, etc. [5] [6]. 2-PP exhibits low acute toxicity in animal experiments [7]. The Japanese government approved its use as a food additive only for citrus fruits in 1977 with the permitted maximum residue level of 10 ppm in whole fruits [8] [9]. The WHO view on the toxicity of 2-PP is as follows [10]: “A health-based value of 1 mg/L can be calculated for 2-PP on the basis of an ADI of 0.4 mg/kg of body weight, based on a NOAEL of 39 mg/kg of body weight per day in a 2-year toxicity study for decreased body weight gain and hyperplasia of the urinary bladder and carcinogenicity of the urinary bladder in male rats, using an uncertainty factor of 100. Because of its low toxicity, however, the health-based value derived for 2-PP is much higher than 2-PP concentrations likely to be found in drinking-water. Under usual conditions, therefore, the presence of 2-PP in drinking-water is unlikely to represent a hazard to human health.”

One of the most widely used methods for determination of total phenols in water samples is visible absorbance measurement following reaction with 4-aminoantipyrine [11] [12]. However, this method can not determine the concentrations of individual phenols. Various separation methods for detection of the phenols described above have been reported, employing GC, HPLC, and capillary electrophoresis with various detection modes, including fluorimetry, mass spectrometry, chemiluminescence, and electrochemical analysis [13]-[18]. Derivatization with a UV-absorbing or fluorescent agent is one of the most useful techniques to improve selectivity and sensitivity, and may make sample clean-up unnecessary. Various reagents such as benzoyl chloride [4], 4-fluoro-7-nitro-2,1,3-benoxadiazole [19], 4-(4,5-diphenyl-1H-imidazol-2-yl) benzoyl chloride [20], coumarin-6-sulfonyl chloride [16], dansyl chloride [21], 2-(9-
carbazole) ethyl chloroformate [22], and 3-chlorocarbonyl-6,7-dimethoxy-1-
methyl-2(1H)-quinoxalinone [23] have been used for analysis of phenol and/or
CPs in biological, food, and environmental samples by means of HPLC with UV
or fluorescence detection.

Various reagents such as 4-fluoro-7-nitro-2,1,3-benzoxadiazole [24] and
4-(N-chloroformylmethyl-N-methylamino)-7-nitro-2,1,3-benzoxadiazole [25]
have been used for analysis of PPs in cosmetic and environmental samples by
means of HPLC with UV and fluorescence detection, respectively. Yang et al.
developed a highly sensitive method of 2-PP determination by HPLC with elec-
trochemical detection, using a microbore column; this afforded a detection limit
of 3.4 pg [9]. GC-mass spectrometry methods for determination of 2-PP after
derivatization with pentafluorobenzoyl bromide and ferrocene carboxylic acid
chloride were applied to beer and citrus fruit samples, respectively [5] [6]. On
the other hand, the WHO does not set guideline values for two positional is o-
mers of 2-PP, 3-phenylphenol (3-PP) and 4-phenylphenol (4-PP), and no me-
thod has been reported for assay of 3-PP or 4-PP. While the previous method
employing HPLC-UV after derivatization with 4-fluoro-7-nitro-2,1,3-benzoxa-
diazole was simple, the reagent is expensive and the system was not appli-
cable for the analysis of PPs, but only phenol and five CPs.

In this paper, we present a simple HPLC-UV method for simultaneous dete-
rmination of phenol, five CPs (2-CP, 4-CP, 2,4-DCP, 2,6-DCP, and 2,4,6-TCP),
and three PPs (2-PP, 3-PP, and 4-PP) in tap water after pre-column derivatiza-
tion with 4-nitrobenzoyl chloride (4-NB-Cl). The derivatization scheme is
shown in Figure 1.

2. Materials and Methods

2.1. Materials

Phenol, 2-CP, 4-CP, 2,4-DCP, 2,6-DCP, 2,4,6-TCP, and general reagents were
obtained from Wako Pure Chemical Industries (Osaka, Japan). 2-PP and 4-PP
were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). 3-PP and
4-NB-Cl were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo). Tap

![Figure 1. Derivatization of Phenol, five CPs and three PPs with 4-NB-Cl.](image-url)
water was collected from our laboratory.

2.2. Chromatographic Conditions

The HPLC system consisted of a model LC10-ATvp pump (Shimadzu, Kyoto, Japan), a Rheodyne injection valve (Cotati, CA, U.S.A.) with a 50-μL loop, and a model SPD-10Avp UV detector (Shimadzu) operating at 280 nm. The HPLC column (Cholesterol, Nacalai tesque, Kyoto) was 150 × 3.0 mm i.d., containing 5 μm particles. Quantification of peaks was performed using a Chromatopac Model C-R8A integrator (Shimadzu). The mobile phase was prepared by the addition of acetonitrile (700 mL) to 300 mL of Milli-Q water containing trifluoroacetic acid (0.1 v/v%). The samples were eluted from the column at room temperature at a flow rate of 0.43 mL/min.

2.3. Derivatization

Ultrapure water was from a Milli-Q water purification system (Simplicity® UV, Millipore Corporation, Bedford, MA, U.S.A.). Standard samples of phenol, five CPs, and three PPs were dissolved in Milli-Q water, acetone, and methanol, respectively, to obtain solution concentrations of 1 g/L. The standard mixture was prepared by dilution as required with Milli-Q water. Borate buffer (0.1 M) was adjusted to various pH values by the addition of NaOH. Borate buffer (100 μL) was added to Milli-Q water (100 μL), and diluted standard samples (100 μL; 0, 0.02, 0.04, 0.06, 0.12, 0.36, 0.6, and 0.9 mg/L) were added to the mixture. Then, 4-NB-Cl solution in acetonitrile (2 mg/mL, 100 μL) was added. The mixture was vortexed and allowed to react at 50°C, then an aliquot (50 μL) was taken and injected into the HPLC system.

2.4. Application to Water Samples

Tap water (100 μL) instead of Milli-Q water (100 μL) was analyzed in the same manner as described above. Relative recovery was expressed as the ratio of the calibration curve prepared from a water sample spiked with the standard sample to the standard calibration curve prepared as described above. Relative recovery data were used to assess the accuracy of the method.

3. Results and Discussion

3.1. Reaction Time Courses of Phenol, Five CPs, and Three PPs with 4-NB-Cl

For the time course study, the reaction time was set at 0.5, 1, 2, 4, 6, and 10 min (Figure 2). Phenol, five CPs, and three PPs (100 μL, each 0.36 mg/L), borate buffer (100 μL, pH 8.5), and 4-NB-Cl (100 μL, 2 mg/mL) were added to Milli-Q water (100 μL) and each solution was left to stand for the appropriate time. All derivatizations reached a plateau at 0.5 or 1 min. However, chromatograms of the reaction mixture at 0.5 min showed a remarkable peak at 4.4 min (data not shown), which might interfere with the peak of 4-NB-phenol. Thus, the derivatization time of 1 min was selected.
Standard samples (each 0.36 mg/L) were reacted with 4-NB-Cl in borate buffer at pH 8.5 at 50°C. ○, 4-NB-phenol; △, 4-NB-4-CP; □, 4-NB-2-CP; ◇, 4-NB-2,4-DCP; ×, 4-NB-2,6-DCP; +, 4-NB-2,4,6-TCP; ●, 4-NB-2-PP; ▲, 4-NB-3-PP; ■ 4-NB-4-PP.

Figure 2. Time courses of formation of 4-NB derivatives of phenol, five CPs, and three PPs.

3.2. pH Dependency of Derivatization of Phenol, Five CPs, and Three PPs with 4-NB-Cl
pH Dependency (pH 8 to 9.5) was examined at the derivatization time of 1 min (Figure 3). Peak areas of derivatives, except the phenol derivative, showed little variation in the range of pH 8 or 8.25 to 9. However, the peak area of 4-NB-phenol at pH 8 was markedly reduced because of interference from the peak at 4.4 min. Therefore, pH 8.5 was selected for the derivatization buffer.

3.3. Chromatogram
Figure 4 shows typical chromatograms obtained from (a) blank and (b) a standard mixture (each 0.36 mg/L) at pH 8.5 for 1 min. The retention times of 4-NB-phenol, 4-NB-2-CP, 4-NB-4-CP, 4-NB-2-PP, 4-NB-2,6-DCP, 4-NB-2,4-DCP, 4-NB-3-PP, 4-NB-4-PP, and 4-NB-2,4,6-TCP were 4.9, 6.0, 6.5, 7.4, 8.1, 9.2, 10.2, 12.2, and 13.6 min, respectively. The running time was 15 min. Our preliminary test showed that 4-NB-2-PP and 4-NB-2,6-DCP were co-eluted (8.0 min) from a C18 column (C18-MS-II, Nakalai tesque, 150×3.0 mm i.d., containing 5 μm particles) under the same HPLC conditions. In addition, the peaks of 4-NB-3-PP (9.7 min) and 4-NB-4-PP (10.0 min) were much closer together than in the case of analysis using the Cholesterol column. Thus, the Cholesterol column was found to be more useful for simultaneous determination of the nine tested phenols.

3.4. Standard Curves of Phenol, Five CPs, and Three PPs
The standard curves of phenol, five CPs, and three PPs were constructed by
Standard samples (each 0.36 mg/L) were reacted with 4-NB-Cl in borate buffer at pH 8.5 at 50˚C. (○), 4-NB-phenol; (△), 4-NB-2-CP; (□), 4-NB-4-CP; (◇), 4-NB-2,4-DCP; (+), 4-NB-2,4,6-TCP; (●), 4-NB-2-PP; (▲), 4-NB-3-PP; (■) 4-NB-4-PP.

Figure 3. pH Dependency of the formation of 4-NB derivatives of phenol, five CPs, and three PPs.

Figure 4. Typical chromatograms of blank (a) and standard sample ((b), each 0.36 mg/L) after derivatization with 4-NB-Cl.
plotting integrated peak area vs. concentration. The calibration data are summarized in Table 1. Plots were all linear in the range of 0.02 ~ 0.12 to 0.9 mg/L, with \( r^2 \) values \( \geq 0.9928 \). The values of the lower limit of quantification were the lowest concentration on the standard curve. Their values were 0.02, 0.06, 0.02, 0.06, 0.12, 0.04, 0.04, 0.04, and 0.002 mg/L for phenol, 2-CP, 4-CP, 2,6-DCP, 2,4-DCP, 2,4,6-TCP, 2-PP, 3-PP, and 4-PP, respectively. The lower limits of detection for phenol, 2-CP, and 4-CP were estimated as the concentrations giving a detectable peak, since these peaks were located close to a large blank peak. The lower limits of detection for 2,4-DCP, 2,6-DCP, 2,4,6-TCP, 2-PP, 3-PP, and 4-PP were taken to be the concentrations giving a signal-to-noise ratio of 3:1. The lower limits of detection were 0.006 (75 pg), 0.02 (250 pg), 0.006 (75 pg), 0.02 (250 pg), 0.05 (625 pg), 0.02 (250 pg), 0.01 (125 pg), 0.01 (125 pg), and 0.008 (100 pg) mg/L for phenol, 2-CP, 4-CP, 2,6-DCP, 2,4-DCP, 2,4,6-TCP, 2-PP, 3-PP, and 4-PP, respectively. Values in parenthesis are the detection limits in terms of absolute amounts. The sensitivity for phenol and CPs was slightly inferior to that in our previous study (absolute amount of 67 to 167 pg) [19]. In addition, the detection limit of 2-PP (125 pg) represents only moderate sensitivity compared with previous reports (range of 3.4 to 350 pg) [8] [9] [26] [27]. Detection limits of 3-PP and 4-PP have not been reported.

### 3.5. Precision and Accuracy

Precision and accuracy for intra-day and inter-day assays of these derivatives are shown in Table 2 and Table 3. In the intra-day assay, the range of standard deviation was within 2.6% to 11.1% of the mean. Recoveries were within the range of 87.5% to 105.2%. In the inter-day assay, the range of standard deviation was within 4.2% to 12.0% of the mean. Recoveries were within the range of 88.5% to 104.1%.

### 3.6. Environmental Analysis

The described method was used to determine phenol, five CPs, and three PPs in tap water and spiked tap water. As shown in Table 4, the levels of phenol, five

<table>
<thead>
<tr>
<th>Compound</th>
<th>Slope</th>
<th>Intercept</th>
<th>Concentration range</th>
<th>( r^2 )</th>
<th>Lower limit of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>148</td>
<td>+0.150</td>
<td>0.02 to 0.9 mg/L</td>
<td>0.9986</td>
<td>0.006 mg/L (75 pg)</td>
</tr>
<tr>
<td>2-CP</td>
<td>123</td>
<td>+0.813</td>
<td>0.06 to 0.9 mg/L</td>
<td>0.9959</td>
<td>0.02 mg/L (250 pg)</td>
</tr>
<tr>
<td>4-CP</td>
<td>141</td>
<td>+0.151</td>
<td>0.02 to 0.9 mg/L</td>
<td>0.9989</td>
<td>0.006 mg/L (75 pg)</td>
</tr>
<tr>
<td>2,6-DCP</td>
<td>77.7</td>
<td>+0.485</td>
<td>0.06 to 0.9 mg/L</td>
<td>0.9969</td>
<td>0.02 mg/L (250 pg)</td>
</tr>
<tr>
<td>2,4-DCP</td>
<td>90.5</td>
<td>+1.68</td>
<td>0.12 to 0.9 mg/L</td>
<td>0.9928</td>
<td>0.05 mg/L (625 pg)</td>
</tr>
<tr>
<td>2,4,6-TCP</td>
<td>56.4</td>
<td>+0.211</td>
<td>0.04 to 0.9 mg/L</td>
<td>0.9975</td>
<td>0.02 mg/L (250 pg)</td>
</tr>
<tr>
<td>2-PP</td>
<td>82.2</td>
<td>+0.142</td>
<td>0.04 to 0.9 mg/L</td>
<td>0.9993</td>
<td>0.01 mg/L (125 pg)</td>
</tr>
<tr>
<td>3-PP</td>
<td>78.7</td>
<td>+0.209</td>
<td>0.04 to 0.9 mg/L</td>
<td>0.9945</td>
<td>0.01 mg/L (125 pg)</td>
</tr>
<tr>
<td>4-PP</td>
<td>141</td>
<td>+0.141</td>
<td>0.02 to 0.9 mg/L</td>
<td>0.9981</td>
<td>0.008 mg/L (100 pg)</td>
</tr>
</tbody>
</table>

Values in parenthesis are absolute amounts (pg).
### Table 2. Intra-day assay reproducibility for determination of phenol, five CPs, and three PPs.

<table>
<thead>
<tr>
<th>Compound (mg/L)</th>
<th>Measured (mg/L, Mean ± S.D., n = 5)</th>
<th>C.V. (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>0.02 0.0178 ± 0.0017</td>
<td>9.6</td>
<td>89.0</td>
</tr>
<tr>
<td></td>
<td>0.9 0.857 ± 0.027</td>
<td>3.2</td>
<td>95.2</td>
</tr>
<tr>
<td>2-CP</td>
<td>0.06 0.0533 ± 0.0041</td>
<td>7.7</td>
<td>88.8</td>
</tr>
<tr>
<td></td>
<td>0.9 0.901 ± 0.032</td>
<td>3.6</td>
<td>100.1</td>
</tr>
<tr>
<td>4-CP</td>
<td>0.02 0.0175 ± 0.0018</td>
<td>10.3</td>
<td>87.5</td>
</tr>
<tr>
<td></td>
<td>0.9 0.947 ± 0.030</td>
<td>3.2</td>
<td>105.2</td>
</tr>
<tr>
<td>2,6-DCP</td>
<td>0.06 0.0552 ± 0.0049</td>
<td>8.9</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td>0.9 0.919 ± 0.030</td>
<td>3.3</td>
<td>102.1</td>
</tr>
<tr>
<td>2,4-DCP</td>
<td>0.12 0.114 ± 0.010</td>
<td>8.8</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>0.9 0.866 ± 0.025</td>
<td>2.9</td>
<td>96.2</td>
</tr>
<tr>
<td>2,4,6-TCP</td>
<td>0.04 0.0359 ± 0.0040</td>
<td>11.1</td>
<td>89.8</td>
</tr>
<tr>
<td></td>
<td>0.9 0.839 ± 0.031</td>
<td>3.7</td>
<td>93.2</td>
</tr>
<tr>
<td>2-PP</td>
<td>0.04 0.0366 ± 0.0040</td>
<td>10.9</td>
<td>91.5</td>
</tr>
<tr>
<td></td>
<td>0.9 0.884 ± 0.023</td>
<td>2.6</td>
<td>98.2</td>
</tr>
<tr>
<td>3-PP</td>
<td>0.04 0.0364 ± 0.0037</td>
<td>10.2</td>
<td>91.0</td>
</tr>
<tr>
<td></td>
<td>0.9 0.914 ± 0.037</td>
<td>4.0</td>
<td>101.6</td>
</tr>
<tr>
<td>4-PP</td>
<td>0.02 0.0184 ± 0.0017</td>
<td>9.2</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td>0.9 0.920 ± 0.027</td>
<td>2.9</td>
<td>102.2</td>
</tr>
</tbody>
</table>

### Table 3. Inter-day assay reproducibility for determination of phenol, five CPs, and three PPs.

<table>
<thead>
<tr>
<th>Compound (mg/L)</th>
<th>Measured (mg/L, Mean ± S.D., n = 5)</th>
<th>C.V. (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>0.02 0.0177 ± 0.0018</td>
<td>10.2</td>
<td>88.5</td>
</tr>
<tr>
<td></td>
<td>0.9 0.850 ± 0.039</td>
<td>4.6</td>
<td>94.4</td>
</tr>
<tr>
<td>2-CP</td>
<td>0.06 0.0538 ± 0.0054</td>
<td>10.0</td>
<td>89.7</td>
</tr>
<tr>
<td></td>
<td>0.9 0.924 ± 0.041</td>
<td>4.4</td>
<td>102.7</td>
</tr>
<tr>
<td>4-CP</td>
<td>0.02 0.0176 ± 0.0020</td>
<td>11.4</td>
<td>88.0</td>
</tr>
<tr>
<td></td>
<td>0.9 0.925 ± 0.045</td>
<td>4.9</td>
<td>102.8</td>
</tr>
<tr>
<td>2,6-DCP</td>
<td>0.06 0.0536 ± 0.0055</td>
<td>10.3</td>
<td>89.3</td>
</tr>
<tr>
<td></td>
<td>0.9 0.932 ± 0.040</td>
<td>4.3</td>
<td>103.6</td>
</tr>
<tr>
<td>2,4-DCP</td>
<td>0.12 0.109 ± 0.013</td>
<td>11.9</td>
<td>90.8</td>
</tr>
<tr>
<td></td>
<td>0.9 0.902 ± 0.038</td>
<td>4.2</td>
<td>100.2</td>
</tr>
<tr>
<td>2,4,6-TCP</td>
<td>0.04 0.0354 ± 0.0040</td>
<td>11.3</td>
<td>88.5</td>
</tr>
<tr>
<td></td>
<td>0.9 0.866 ± 0.045</td>
<td>5.2</td>
<td>96.2</td>
</tr>
<tr>
<td>2-PP</td>
<td>0.04 0.0368 ± 0.0043</td>
<td>11.7</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td>0.9 0.859 ± 0.057</td>
<td>6.6</td>
<td>95.4</td>
</tr>
<tr>
<td>3-PP</td>
<td>0.04 0.0358 ± 0.0043</td>
<td>12.0</td>
<td>89.5</td>
</tr>
<tr>
<td></td>
<td>0.9 0.937 ± 0.061</td>
<td>6.5</td>
<td>104.1</td>
</tr>
<tr>
<td>4-PP</td>
<td>0.02 0.0182 ± 0.0021</td>
<td>11.5</td>
<td>91.0</td>
</tr>
<tr>
<td></td>
<td>0.9 0.914 ± 0.058</td>
<td>6.3</td>
<td>101.6</td>
</tr>
</tbody>
</table>
Table 4. Levels of phenol, five CPs, and three PPs in tap water, and relative recovery values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration in tap water (%), mean ± S.D., n = 4</th>
<th>Relative recovery</th>
<th>( r^2 ) (Average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>N.D.</td>
<td>94.4 ± 6.2</td>
<td>0.9993</td>
</tr>
<tr>
<td>2-CP</td>
<td>N.D.</td>
<td>105.9 ± 3.9</td>
<td>0.9972</td>
</tr>
<tr>
<td>4-CP</td>
<td>N.D.</td>
<td>109.2 ± 8.5</td>
<td>0.9982</td>
</tr>
<tr>
<td>2,6-DCP</td>
<td>N.D.</td>
<td>94.9 ± 4.2</td>
<td>0.9961</td>
</tr>
<tr>
<td>2,4-DCP</td>
<td>N.D.</td>
<td>109.0 ± 8.7</td>
<td>0.9932</td>
</tr>
<tr>
<td>2,4,6-TCP</td>
<td>N.D.</td>
<td>107.3 ± 6.8</td>
<td>0.9964</td>
</tr>
<tr>
<td>2-PP</td>
<td>N.D.</td>
<td>104.3 ± 5.6</td>
<td>0.9989</td>
</tr>
<tr>
<td>3-PP</td>
<td>N.D.</td>
<td>107.0 ± 6.2</td>
<td>0.9941</td>
</tr>
<tr>
<td>4-PP</td>
<td>N.D.</td>
<td>107.5 ± 5.3</td>
<td>0.9956</td>
</tr>
</tbody>
</table>

N.D., not determined.

CPs, and three PPs in tap water were below the lower limit of quantification. Calibration curves prepared from tap water samples spiked with phenol, five CPs, and three PPs showed linear relationships between concentration and peak response, with \( r^2 \geq 0.9932 \), and the relative recovery values were 94.4% to 109.2%. These results indicate that our method is capable of monitoring tap water for contamination with phenol, CPs, and/or PPs.

4. Conclusion

We have developed an HPLC-UV method using a Cholesterol column for simultaneous determination of nine compounds (phenol, 2-CP, 4-CP, 2,4-DCP, 2,6-DCP, 2,4,6-TCP, 2-PP, 3-PP, and 4-PP) in tap water by using 4-NB-Cl as a UV-labeling reagent, without complicated sample clean-up. The fast derivatization, the inexpensive reagent, and the short running time were shown in this paper. The presented system is simple and suitable for monitoring or routine testing of tap water for contamination with the test compounds (phenol, five CPs, and/or three PPs), i.e., for assessing compliance with official guidelines.

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