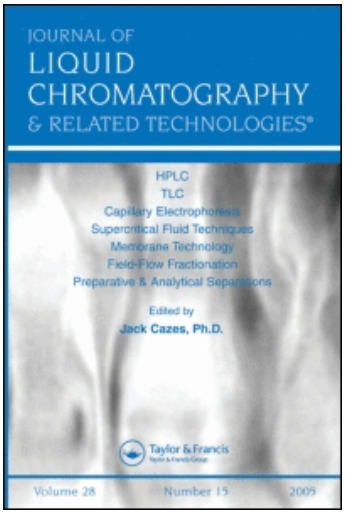

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HPLC-UV Analysis of Phenol and Chlorophenols in Water After Precolumn Derivatization with 4-Fluoro-7-nitro-2,1,3-benzoxadiazole

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Abstract: Chlorophenols (2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, and 2,4,6-trichlorophenol) are present in drinking water and tap water as a result of disinfection processes employing chlorination. In this study, the levels of phenol and the above chlorophenols in water (100 μL aliquots) were simultaneously analyzed by HPLC-UV (380 nm) after precolumn derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). Standard curves were obtained after derivatization with NBD-F in borate buffer (pH 8.5) at room temperature for 5 min. The six NBD-labeled compounds were well separated in less than 20 min. Plots were linear in the range of 0.01∼0.03 to 0.5 mg/L, with r^2 value ≥0.9953, for all compounds. The lower limits of detection were 0.004 to 0.01 mg/L (signal-to-noise ratio of 3:1). The coefficients of variation were less than 5.7%. The recovery values of phenol and chlorophenols from tap water and natural mineral water spiked with the standard mixture were satisfactory. While the levels of phenol and chlorophenols in water samples tested were below the lower limit of determination, our method is expected to be useful for identifying environmental water samples that are contaminated with phenol and chlorophenols, i.e., for testing whether or not official guidelines are met.

Keywords: 4-Fluoro-7-nitro-2,1,3-benzoxadiazole, Chlorophenol, Derivatization, Phenol, UV

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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, 4-chlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, and 2,4,6-trichlorophenol are malodorous even at very low concentration 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 WHO guideline value for 2,4,6-trichlorophenol is 0.2 mg/L, and concentrations of chlorophenols 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.[2–4] 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. One of the most widely used methods for determination of total phenols in water samples is visible absorbance measurement following reaction with 4-aminoantipyrine.[5,6] However, this method can not determine the concentrations of individual phenols. Various separation methods for detection of phenols have been reported, employing GC, HPLC, and capillary electrophoresis with various detection modes, including fluorimetry, mass spectrometry, chemiluminescence, and electrochemical analysis.[7–12]

Derivatization with a UV-absorbing or fluorescent agent is one of the most useful techniques to improve selectivity and sensitivity, and may make sample cleanup unnecessary. However, previous methods for determination of phenol in river water and wine by HPLC-UV after derivatization with benzoyl chloride, and for simultaneous analysis of phenol and chlorophenols in urine by HPLC fluorescence detection after derivatization with 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride, required sample pretreatment.[13,14] There is one report of HPLC fluorescence detection of phenol and chlorophenols in various environmental waters after derivatization with coumarin-6-sulfonyl chloride, in which simple filtration was sufficient for sample cleanup.[10]

For routine detection of phenols in water to ensure water quality, it would be preferable to use an HPLC-UV system, since this equipment is widely available; further, for the sake of speed and low cost, the requirement for sample cleanup should be minimal. 4-Fluoro-7-nitro-2,1,

Phenol and chlorophenols	
Phenol	$R_1=R_2=R_3=H$
2-Chlorophenol	$R_1=Cl, R_2=R_3=H$
4-Chlorophenol	$R_1=R_3=H, R_2=Cl$
2,4-Dichlorophenol	$R_1=R_2=Cl, R_3=H$
2,6-Dichlorophenol	$R_1=R_3=Cl, R_2=H$
2,4,6-Trichlorophenol	$R_1=R_2=R_3=Cl$

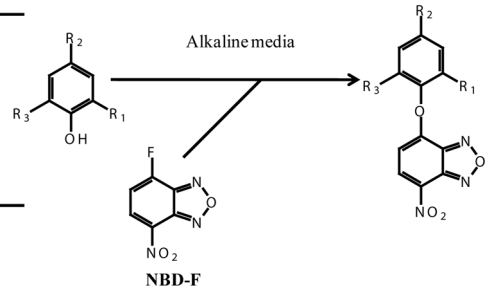

Figure 1. Derivatization of phenol and chlorophenols with NBD-F.

3-benzoxadiazole (NBD-F) has been used as a fluorescent labeling agent for primary and secondary amino groups for HPLC fluorescence detection.[15–19] On the other hand, Toyo'oka et al. used NBD-F as a UV labeling reagent reactive with the phenolic hydroxyl group of *N*-acetyltyrosine.[20] In this paper, we present a HPLC-UV method for simultaneous determination of phenol and five chlorophenols in water after precolumn derivatization with NBD-F, with simple sample cleanup by filtration. The derivatization scheme is shown in Figure 1.

EXPERIMENTAL

Reagents

Phenol, 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 2,4,6-trichlorophenol, NBD-F, and general reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan). Tap water was collected from our laboratory, and natural bottled mineral water from France was purchased from a market.

Equipment

The HPLC system consisted of a model LC10-ATvp pump (Shimadzu, Kyoto, Japan), a Rheodyne injection valve (Cotati, CA, USA) with a 50-μL loop, and a model SPD-10Avp UV detector (Shimadzu) operating at 380 nm. The HPLC column (C_{18}-MS-II, Nacalai tesque, Kyoto) was 150×3.0 mm i.d., containing 5 μm particles of C_{18} packing material. Quantification of peaks was performed using a Chromatopac Model C-R8A integrator (Shimadzu). The mobile phase was prepared by the addition of acetonitrile (600 mL) to 400 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.

Derivatization

Ultrapure water was from a Milli-Q water purification system (Simplicity® UV, Millipore Corporation, Bedford, MA, USA). Standard samples of phenol and chlorophenols were dissolved in Milli-Q water and acetone, 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 pH 8.5 by the addition of NaOH. Borate buffer (100 μL) was added to diluted standard samples (100 μL; 0, 0.01, 0.02, 0.03, 0.05, 0.1, 0.2, 0.5 mg/L). NBD-F solution in acetonitrile (1 mg/mL, 100 μL) was added and vortexed. The mixture was allowed to react for 5 min at room temperature, then an aliquot (50 μL) was injected into the HPLC system.

Application to Water Samples and Evaluation of Relative Recovery

Water samples spiked with standard samples (lower limit of quantification, 0.05, 0.1, and 0.2 mg/L) were passed through 0.45 μm filters (Cosmonice Filter S, Nacalai tesque) to remove suspended substances and analyzed quickly. It was confirmed, that the filtration procedure had no effect on the recovery by analyzing a sample spiked with standards before and after filtration; no significant difference in the recovery was observed.

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.

RESULTS AND DISCUSSION

Derivatization of Phenol and Five Chlorophenols with NBD-F

For the time course study, the reaction time was set at 1.5, 3, 5, 10, 20, and 30 min. Phenol and five chlorophenols (100 μL, each 0.5 mg/L), borate buffer (100 μL, pH 8.5) and NBD-F (100 μL, 1 mg/mL) were mixed as described in Materials and Methods. The derivatizations of phenol, 2-chlorophenol, and 4-chlorophenol reached a plateau at 3 min, and those of 2,4-dichlorophenol, 2,6-dichlorophenol, and 2,4,6-trichlorophenol reached a plateau at 5 min (Figure 2).

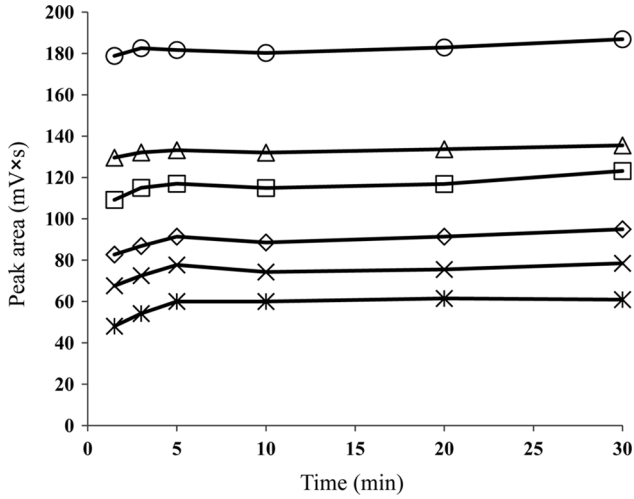

Figure 2. Time courses on the formation of phenol and chlorophenols derivative with NBD-F. Standard sample (each 0.5 mg/L) was reactive to NBD-F in borate buffer at pH 8.5. (◯), NBD-phenol; (△), NBD-4-chlorophenol; (□), NBD-2-chlorophenol; (◇), NBD-2,4-dichlorophenol; (×), NBD-2,6-dichlorophenol; (∗), NBD-2,4,6-trichlorophenol.

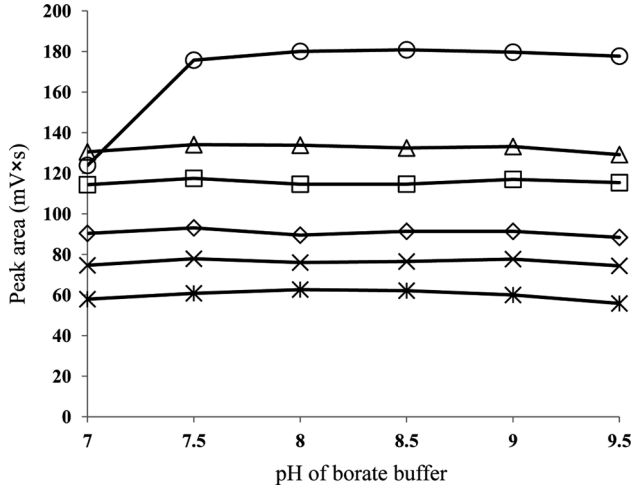

Figure 3. pH Dependency on the formation of phenol and chlorophenols derivatives with NBD-F. Standard sample (each 0.5 mg/L) was reactive to NBD-F for 5 min in various borate buffers. (◯), NBD-phenol; (△), NBD-4-chlorophenol; (□), NBD-2-chlorophenol; (◇), NBD-2,4-dichlorophenol; (×), NBD-2,6-dichlorophenol; (∗), NBD-2,4,6-trichlorophenol.

Next, pH dependency (pH 7.0 to 9.5) was examined at the derivatization time of 5 min. Peak areas of NBD-phenol at pH 7.0 and NBD-2,4,6-trichlorophenol at pH 7.0 and 9.5 were 70 to 90% of the maximum (Figure 3); all others were more than 95% of the maximum. Thus, the derivatization time of 5 min at pH 8.5 was selected.

Chromatogram

Figure 4 shows typical chromatograms obtained from (A) blank and (B) standard sample (0.5 mg/L). The retention times of NBD-phenol,

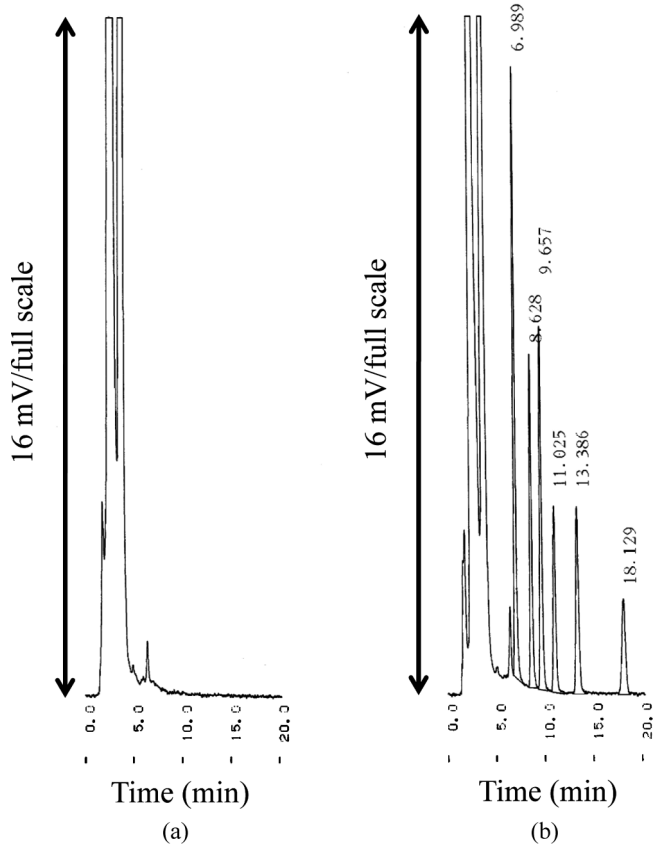

Figure 4. Typical chromatograms of blank (a) and standard sample (b, each 0.5 mg/L) after derivatization with NBD-F. Standard sample (each 0.5 mg/L) was reactive to NBD-F for 5 min at pH 8.5. Retention times (min): 7.0, NBD-phenol; 8.6, NBD-2-chlorophenol; 9.7, NBD-4-chlorophenol; 11.0, NBD-2,6-dichlorophenol; 13.4, NBD-2,4-dichlorophenol; 18.1, NBD-2,4,6-trichlorophenol.

Table 1. Linear correlation parameters

Compounds	Slope	Intercept	Concentration range (mg/L)	r^2
Phenol	357.6	+0.7130	0.01 to 0.5	0.9997
2-Chlorophenol	237.6	+0.2073	0.01 to 0.5	0.9995
4-Chlorophenol	267.0	−0.06987	0.01 to 0.5	0.9995
2,6-Dichlorophenol	154.9	+0.4235	0.02 to 0.5	0.9998
2,4-Dichlorophenol	181.4	−0.2219	0.02 to 0.5	0.9975
2,4,6-Trichlorophenol	124.6	+0.4896	0.03 to 0.5	0.9953

NBD-2-chlorophenol, NBD-4-chlorophenol, NBD-2,6-dichlorophenol, NBD-2,4-dichlorophenol, and NBD-2,4,6-trichlorophenol were 7.0, 8.6, 9.7, 11.0, 13.4, and 18.1 min, respectively.

Table 2. Intra-day assay reproducibility for determination of phenol and chlorophenols

Compounds (mg/L)	Measured (mg/L, Mean ± S.D., $n = 5$)	C.V. (%)	Recovery (%)
Phenol			
0.01	0.0104 ± 0.0005	4.8	104.0
0.05	0.0508 ± 0.0015	3.0	101.6
0.5	0.526 ± 0.016	3.0	105.2
2-Chlorophenol			
0.01	0.0105 ± 0.0005	4.8	105.0
0.05	0.0520 ± 0.0014	2.7	104.0
0.5	0.522 ± 0.020	3.8	104.4
4-Chlorophenol			
0.01	0.00992 ± 0.0005	5.0	99.2
0.05	0.0478 ± 0.0013	2.7	95.6
0.5	0.488 ± 0.014	2.9	97.6
2,6-Dichlorophenol			
0.02	0.0198 ± 0.0010	5.1	99.0
0.05	0.0488 ± 0.0014	2.9	97.6
0.5	0.514 ± 0.016	3.1	102.8
2,4-Dichlorophenol			
0.02	0.0206 ± 0.0011	5.3	103.0
0.05	0.0492 ± 0.0014	2.8	98.4
0.5	0.512 ± 0.014	2.7	102.4
2,4,6-Trichlorophenol			
0.03	0.0312 ± 0.0016	5.1	104.0
0.05	0.0508 ± 0.0016	3.1	101.6
0.5	0.498 ± 0.015	3.0	99.6

Standard Curves of Phenol and Five Chlorophenols

The standard curves of phenol, 2-chlorophenol, 4-chlorophenol, 2,6-dichlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol were constructed by plotting integrated peak area *vs.* concentration. The calibration data are summarized in Table 1. Plots were linear in the range of 0.01∼0.03 to 0.5 mg/L, with r^2 value ≥ 0.9953, for all compounds. The values of the lower limit of quantification were the lowest concentration on the standard curve, and the lower limits of detection were 0.004, 0.006, 0.005, 0.008, 0.006, and 0.01 mg/L for phenol, 2-chlorophenol, 4-chlorophenol, 2,6-dichlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol, respectively (signal-to-noise ratio of 3:1). The sensitivity is about 2- to 80-fold inferior as compared with previous methods.[10,13,14]

Table 3. Inter-day assay reproducibility for determination of phenol and chlorophenols

Compounds (mg/L)	Measured (mg/L, Mean ± S.D., $n = 5$)	C.V. (%)	Recovery (%)
Phenol			
0.01	0.00994 ± 0.00054	5.4	99.4
0.05	0.0522 ± 0.0026	5.0	104.4
0.5	0.504 ± 0.021	4.2	100.8
2-Chlorophenol			
0.01	0.00984 ± 0.00053	5.4	98.4
0.05	0.0472 ± 0.0025	5.3	94.4
0.5	0.502 ± 0.025	5.0	100.4
4-Chlorophenol			
0.01	0.0104 ± 0.0005	4.8	104.0
0.05	0.0488 ± 0.0023	4.7	97.6
0.5	0.478 ± 0.022	4.6	95.6
2,6-Dichlorophenol			
0.02	0.0208 ± 0.0010	4.8	104.0
0.05	0.0516 ± 0.0026	5.0	103.2
0.5	0.516 ± 0.025	4.8	103.2
2,4-Dichlorophenol			
0.02	0.0196 ± 0.0011	5.6	98.0
0.05	0.0504 ± 0.0024	4.8	100.8
0.5	0.514 ± 0.021	4.1	102.8
2,4,6-Trichlorophenol			
0.03	0.0314 ± 0.0018	5.7	104.8
0.05	0.0512 ± 0.0024	4.7	102.4
0.5	0.531 ± 0.026	4.9	106.2

Toyo'oka et al. reported that the NBD derivative of *N*-acetyltyrosine exhibited two UV maxima at 275 and 380 nm.[20] Peak areas of phenol and chlorophenol derivatives (each 0.5 mg/L) monitored at 380 nm were 1.3 to 2.0 fold higher than those monitored at 275 nm. The sensitivity and slope values at 380 nm were similarly higher (data not shown). Therefore, monitoring at 380 nm was selected.

Precision and Accuracy

Precision and accuracy for intra-day and inter-day assays of these derivatives are shown in Tables 2 and 3. In the intra-day assay, the range of standard deviation was within 2.7 to 5.3% of the mean. Recoveries were within the range of 95.6 to 105.2%. In the inter-day assay, the range of standard deviation was within 4.1 to 5.7% of the mean. Recoveries were within the range of 94.4 to 106.2%.

Environmental Analysis

The described method was used to determine phenol and chlorophenols in tap water, spiked tap water, and natural mineral water. As shown in Table 4, the level of phenol and chlorophenols in tap water and natural

Table 4. Levels of phenol and chlorophenols in tap water and natural mineral water, and the relative recovery

Compounds	Concentration in water samples	Relative recovery (%, mean ± S.D., $n = 3$)
Tap water		
Phenol	N.D.	98.2 ± 5.1
2-Chlorophenol	N.D.	95.2 ± 4.4
4-Chlorophenol	N.D.	97.4 ± 4.2
2,6-Dichlorophenol	N.D.	91.9 ± 4.9
2,4-Dichlorophenol	N.D.	107.5 ± 6.8
2,4,6-Trichlorophenol	N.D.	108.0 ± 5.8
Natural mineral water		
Phenol	N.D.	97.6 ± 4.9
2-Chlorophenol	N.D.	98.2 ± 5.8
4-Chlorophenol	N.D.	98.8 ± 4.9
2,6-Dichlorophenol	N.D.	107.0 ± 5.2
2,4-Dichlorophenol	N.D.	106.1 ± 5.5
2,4,6-Trichlorophenol	N.D.	97.6 ± 5.1

N.D., not determined

mineral water were below the lower limit of quantification. Calibration curves prepared from tap water samples spiked with the six phenolic compounds showed linear relationships between concentration and peak response, with $r^2 \geq 0.9979$. The slope of these calibration curves was similar to that of the standard calibration curves, and the relative recovery values were 91.9 to 108.0%. In the case of spiked natural mineral water, the plots were also linear, with $r^2 \geq 0.9947$ for all compounds, and the relative recovery values were 97.6 to 107.0%. These results indicate that our method is capable of monitoring phenol and chlorophenols contamination in tap water and natural mineral water.

CONCLUSION

We have developed a simple HPLC-UV method for simultaneous determination of phenol and chlorophenols in water by using NBD-F as a labeling reagent, without complicated sample cleanup. This system is simple, reproducible, and suitable for routine testing of natural mineral water and tap water for contamination with phenol, 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, and 2,4,6-trichlorophenol, i.e., for testing whether or not official guidelines are met.

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