

Determination of Phenols in Drinking and Bottled Mineral Waters Using Online Solid-Phase Extraction Followed by HPLC with UV Detection

Frank Steiner,¹ Fraser McLeod,¹ Jeff Rohrer,² Susanne Fabel,² Li Lang,³ Xu Qun,³ Chen Jing,³
¹Dionex Corporation, Germering, Germany; ²Dionex Corporation, Sunnyvale, CA, USA; ³Dionex Corporation, Shanghai, China

INTRODUCTION

Phenolic compounds are subject to regulation as water pollutants due to their toxicity. The European Community (EC) Directive specifies a legal tolerance level of 0.5 µg/L for each phenol in water intended for human consumption¹ and Japan's Ministry of Health, Labour, and Welfare specifies a maximum contaminant level (MCL) of 5 µg/L for phenols in drinking water.² The U.S. EPA specifies a MCL of 1 µg/L for pentachlorophenol,³ and eleven common phenols are on the U.S. EPA priority pollutants list.⁴ The structures for these common phenols are shown in Figure 1. The method typically used for determining phenols is gas chromatography (GC) combined with flame ionization detection (FID)^{5,6} or mass spectrometric detection (GC-MS).⁷⁻⁹ However, liquid chromatography (LC) methods combined with UV/DAD,¹⁰ electrochemical,¹¹ and fluorescence¹² detections are finding increased application, particularly due to nonvolatiles in many samples that can poison GC columns.

Method detection limits (MDLs) of LC techniques employing direct injection of samples are too high for the detection of the low levels allowed in natural waters. Therefore, water samples require preconcentration before analysis. Solid-phase extraction (SPE) is one of the most important techniques for sample enrichment, because it overcomes many of the disadvantages of liquid-liquid extraction. Unfortunately, preparing individual samples is time consuming, and a new SPE cartridge must be used for each sample.

The expense of using multiple SPE cartridges and the associated manual labor can be eliminated with online SPE combined with HPLC. This technique delivers a simple, rapid, and accurate means for determining phenols at low concentrations in real samples.^{13,14} The UltiMate® 3000 system was designed to easily execute more advanced HPLC methods, such as parallel LC, 2-D LC, and online SPE/HPLC. An UltiMate 3000 together with an autosampler capable of injecting large volumes can be used to execute an online SPE method to determine phenols in drinking and bottled waters.

This presentation details an online SPE method followed by HPLC with UV detection for determining the 11 phenols specified in U.S. EPA Priority Pollutants List at the concentrations required by world regulatory agencies. Phenols from drinking and bottled waters are trapped on an IonPac® NG1 column, a small polymeric reversed-phase column, then separated on a polar-embedded reversed-phase column, the Acclaim® PA column. This automated method is a cost-effective way to determine phenols in drinking and bottled water samples.

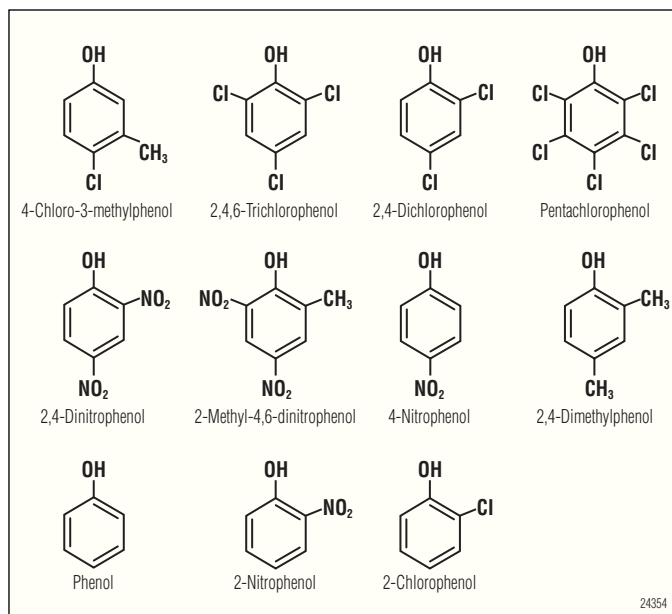


Figure 1. Structures of the 11 phenols specified in the U.S. EPA priority pollutants list.

EQUIPMENT

Dionex UltiMate 3000 HPLC system consisting of:

DGP-3600M dual gradient pump

SRD-3600 solvent rack with integrated vacuum degasser

TCC-3200 Thermostatted Column Compartment with two two-port, six-position (2P-6P) valves

VWD-3400 Variable Wavelength Detector

AS-HV High-Volume Autosampler

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CONDITIONS

Solid-Phase Extraction

Column: IonPac NG1, 5 μ m, 4 \times 35 mm (P/N 039567)

Mobile Phases for SPE (Left Pump):
A. 0.2 mM methane sulfonic acid (MSA)
B. CH₃CN

Flow Rates: Rinse: 1 mL/min with 100% B
Loading: 2 mL/min with 100% A

Phenol Elution: 1 mL/min with 15% B

Inj. Volume: 10 mL

Column Temperature: 40 °C

The total time for on-line SPE is 14 min. For the detailed program see Table 1A.

Analytical

Column: Acclaim PA, 5 μ m, 4.6 \times 150 mm (P/N 061320)

Mobile Phases for Analysis (Right Pump): A. 25 mM HAC / 25 mM NH₄Ac (1.45 : 1, v/v)
B. CH₃CN

Gradient: 25 to 70% B in 17.5 min

Flow Rate: 1 mL/min

Inj. Volume: 10 mL

Temperature: 40 °C

Detection: UV, 280 nm

Total analysis time is 18 min. During SPE, the column is equilibrated for the next separation prior to injection while online SPE is occurring. For the detailed program see Table 1B.

Table 1A. Left Pump Program (Loading Pump Used for SPE) A = 0.2 mM MSA, B = Acetonitrile		
Time (min)	Commands	Comments
Preparation	ValveLeft = 6_1, ValveRight = 6_1	
-14.0	Flow = 1000 [μ L/min] %B = 100.0, %C = 0.0, Curve = 5	Rinse the SPE column (NG1) using 100% CH ₃ CN, about 3 min.
-11.5	Flow = 1000 [μ L/min] %B = 100.0, %C = 0.0, Curve = 5	
-11.0	Flow = 1000 [μ L/min] %B = 1.0, %C = 0.0, Curve = 5	Equilibrate the SPE column.
-8.5	Flow = 2000 [μ L/min] %B = 1.0, %C = 0.0, Curve = 5	Load sample from the loop to SPE column at 2 mL/min, about 5 min.
-3.5	Flow = 2000 [μ L/min] %B = 1.0, %C = 0.0, Curve = 5	
-3.0	Flow = 1000 [μ L/min] %B = 15.0, %C = 0.0, Curve = 5	Wash the SPE column.
0.2	Flow = 0 [μ L/min] %B = 0.0, %C = 0.0, Curve = 5	
3.5	Flow = 200 [μ L/min] %B = 100.0, %C = 0.0, Curve = 5	SPE column switches back to the system. Begin to wash the SPE column to prepare for loading the next sample.

Table 1B. Right Pump Program (Analytical Pump) A = 25 mM HAC/NH ₄ Ac, B = Acetonitrile		
Time (min)	Commands	Comments
Preparation	ValveLeft = 6_1, ValveRight = 6_1	
-14.0	Flow = 200 [μ L/min], %B = 100.0, %C = 0.0, Curve = 5	Wash the analytical column.
-13.0	Flow = 200 [μ L/min], %B = 25.0, %C = 0.0, Curve = 5	
-7.0	Flow = 200 [μ L/min], %B = 25.0, %C = 0.0, Curve = 5	
-5.0	Flow = 1000 [μ L/min], %B = 25.0, %C = 0.0, Curve = 5	Begin to equilibrate the analytical column using initial conditions for 5 min. Injections at 0 min.
17.5	Flow = 1000 [μ L/min], %B = 70.0, %C = 0.0, Curve = 5	17.5 min gradient
18.0	Flow = 1000 [μ L/min], %B = 100.0, %C = 0.0, Curve = 5	Begin the column wash.

SYSTEM SETUP

Figure 2A is a schematic of the devices used for the determination of phenols using online solid-phase extraction (SPE) followed by HPLC with UV detection. The AS-HV has a peristaltic pump that can draw samples from sample bottles through a movable needle. This needle can sample from 15 different 100 mL sample bottles in the sample tray. The movement of the AS-HV is controlled by Chromeleon software. The AS-HV uses the left valve of the TCC-3200 as a sample valve and the right valve as an online SPE switching valve. Figure 2B shows the diagram for programming the large volume injection using the AS-HV. The program for the AS-HV is listed in Table 2. Tables 1A and 1B list the programs for the left (SPE) and right (analytical) for the left (SPE) and right (analytical) pumps in the UltiMate 3000 dual gradient device.

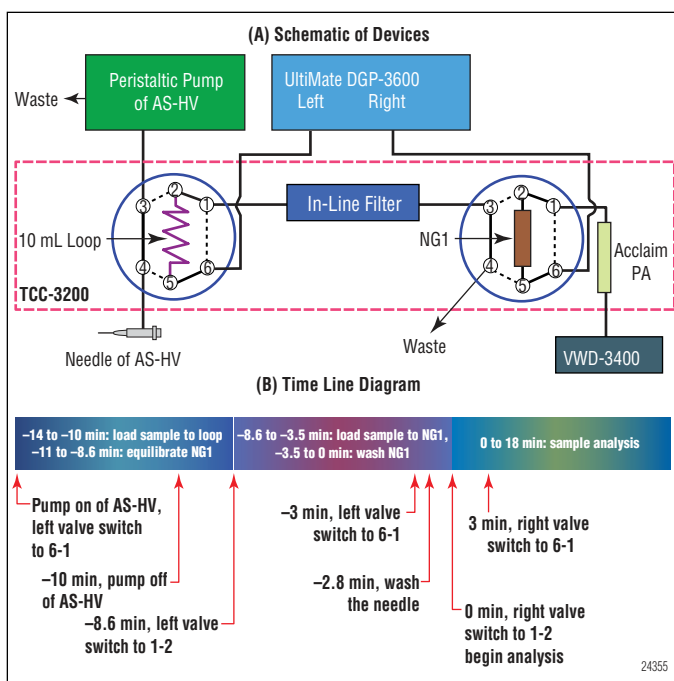


Figure 2. A) Schematic of devices for determination of phenols using online solid-phase extraction (SPE) followed by HPLC with UV detection. B) Time line diagram for programming the high-volume injection using the AS-HV.

Table 2. AS-HV Program		
Time (min)	Commands	Comments
Preparation	Y_Axis = AIM_sampler.position X_Axis = AIM_sampler.position Needle = 157, Go To Position	Find position from CM sequence. Set the needle's height and enter the sample bottle.
-14.0	Pump On	Begin to load sample from the bottle. The flow rate of the peristaltic pump is about 3.3 mL/min.
-10.0	Pump Off Needle Home	End sample loading. After sample loading, sample loop switches inline with the SPE column.
-2.8	AIM Sampler, Wash = On, Pump On	Wash the sampling needle and the sample loop in preparation for the next injection.
3.0	Pump Off, Needle Home	End of AS-HV wash.

SAMPLE PREPARATION

For this analysis, bottled water was purchased from a supermarket close to the Dionex Shanghai Applications Lab located in the Pudong District, Shanghai. The sample was prepared by filtering 495 mL of the water through 0.45 μm filters into four 500-mL bottles and adding 5 mL methanol and 56 μL MSA to each. The final concentration of MSA in the samples was approximately 2 mM.

Spiked samples were prepared from the above solutions.

RESULTS AND DISCUSSION

Using the system setup and methods described, it was possible to concentrate all phenols, separate them, and reliably detect down to extremely low levels. The results are described in more detail below.

Reproducibility, Detection Limits, and Linearity

The reproducibility was estimated by making seven replicate injections of the 2 $\mu\text{g/L}$ calibration standard. Table 3 summarizes the retention time and peak area precision data. The method detection limits (MDLs) of the phenols are also listed in Table 4, as are the MDLs reported for the GC method in U.S. EPA Method 604. The MDLs of the on-line SPE-HPLC method are similar to and in most cases better than those achieved using GC, without the labor and cost of liquid/liquid extraction or manual SPE.

Calibration linearity for the determination of phenols was investigated by making replicate injections of a mixed standard of phenols prepared at six different concentrations. The external standard method is used in EPA Method 604. Therefore, we used it to calculate the calibration curve and for sample analysis. Table 4 lists the data from the calibration as reported by Chromeleon.

Table 3. Retention Time Reproducibility, Peak Area Reproducibility, and Comparison of Detection Limits for the 11 Phenols on the U. S. EPA Priority Pollutants List					
Phenol	RT RSD ^a (%)	Area RSD ^a (%)	MDL ^b ($\mu\text{g/L}$)	MDL ($\mu\text{g/L}$) obtained by GC-FID in EPA 604	MDL ($\mu\text{g/L}$) obtained by GC-ECD in EPA 604
2,4-Dinitrophenol	0.292	1.358	0.46	13.0	0.63
Phenol	0.240	5.584	0.87	0.14	2.2
4,6-Dinitro-2-methylphenol	0.164	0.647	0.40	16.0	not detected
4-Nitrophenol	0.155	0.432	0.42	2.8	0.70
2-Chlorophenol	0.122	1.659	0.41	0.31	0.58
2-Nitrophenol	0.092	1.487	0.41	0.45	0.77
2,4-Dimethylphenol	0.089	0.462	0.30	0.32	0.68
4-Chloro-3-methylphenol	0.085	0.477	0.31	0.36	1.8
2,4-Dichlorophenol	0.072	0.731	0.08	0.39	not detected
2,4,6-Trichlorophenol	0.056	0.717	0.20	0.64	0.58
Pentachlorophenol	0.064	8.599	0.93	7.40	0.59

^aSeven injections of the 2 $\mu\text{g/L}$ working standard solution.

^bThe single-sided Student's *t* test method (at the 99% confidence limit) was used for estimating MDL, where the standard deviation (SD) of the peak area of seven injections is multiplied by 3.14 (at $n = 7$) to yield the MDL.

Table 4. Calibration Data and Linearity of the 11 Phenols

Phenol	r	RSD (%)
2,4-Dinitrophenol	0.9998	1.73
Phenol	0.9984	4.29
4,6-Dinitro-2-methylphenol	0.9998	1.69
4-Nitrophenol	0.9997	1.79
2-Chlorophenol	0.9996	2.22
2-Nitrophenol	0.9992	3.03
2,4-Dimethylphenol	0.9999	1.33
4-Chloro-3-methylphenol	0.9998	1.42
2,4-Dichlorophenol	0.9998	1.33
2,4,6-Trichlorophenol	0.9999	1.28
Pentachlorophenol	0.9965	6.07

Sample Analysis

To achieve satisfactory chromatography of phenols in the tap and mineral water samples, these samples should be acidified to approximately pH 3.5 prior to analysis. Figure 3 shows the chromatograms of spiked mineral water sample acidified to pH 7 and pH 3 with MSA, respectively. The peak shapes of 2,4-dinitrophenol, 4,6-dinitro-2-methylphenol, and 4-nitrophenol are superior at pH 3.

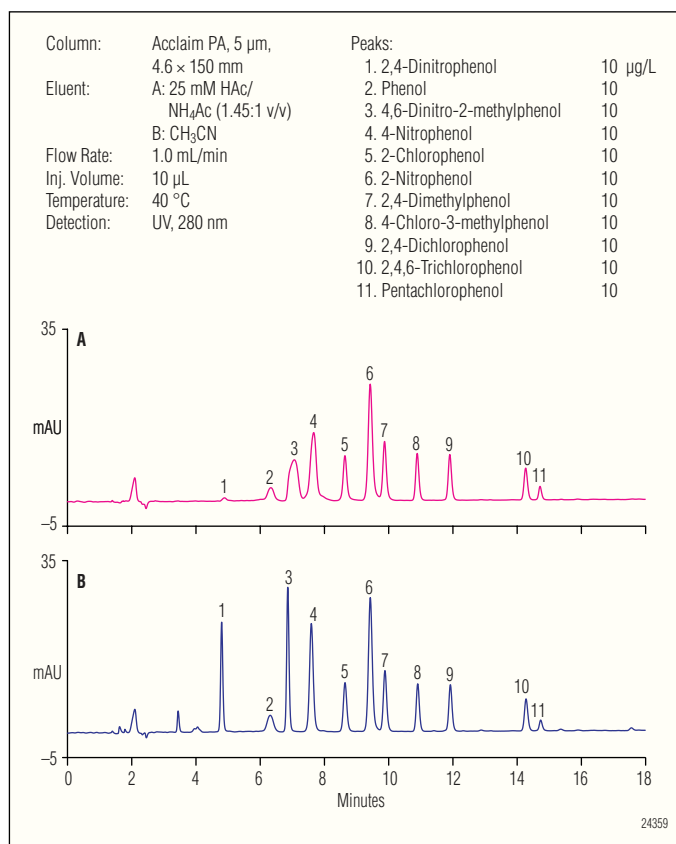


Figure 3. Chromatograms of bottled mineral drinking water 1 spiked with 10 μ g/L phenols and acidified with MSA to A) pH 7, and B) pH 3.

For different water samples, the amount of acid required to achieve a pH < 4.5 varies. For example, 6 μ L MSA (about 0.2 mM final concentration) was added to the 500 mL pure distilled water sample solution (495 mL distilled water + 5 mL methanol) to yield a pH of approximately 3.9. For the tap water and mineral water samples, much more MSA was needed because these samples contain ions that are capable of buffering the MSA, most notably bicarbonate (Table 5). Therefore, approximately 56 μ L MSA (about 2 mM final concentration) was added to the tap and mineral water samples to achieve pH values ranging from 2.5 to 4.5.

Bottled Mineral Drinking Water

Two brands of bottled mineral drinking water were analyzed. Table 5 shows the contents listed on the labels of each. Figures 4 and 5 show chromatograms of the bottled mineral water samples and the same samples spiked with phenols. The results are summarized in Table 6. Low concentrations of two phenols were detected in the unspiked mineral water 2 sample and a low concentration of one phenol in the unspiked mineral water 1. Good recoveries were obtained for all eleven phenols.

Table 5. Listed Amounts of Ions in Bottled Mineral Drinking Waters

Labeled contents	Bottled mineral drinking water 1 (mg/L)	Bottled mineral drinking water 2 (mg/L)
Na ⁺	≥ 0.8	4–12
K ⁺	≥ 0.35	0.3–1.0
Ca ²⁺	≥ 4	not reported
Mg ²⁺	≥ 0.5	0.3–0.5
Zn ²⁺	not reported	0.25
Sr ²⁺	not reported	0.14
HSiO ₂	≥ 1.8	71.6
HCO ₃ ⁻	not reported	14
pH (25 $^{\circ}$ C)	7.35 \pm 0.5	7.0–8.0

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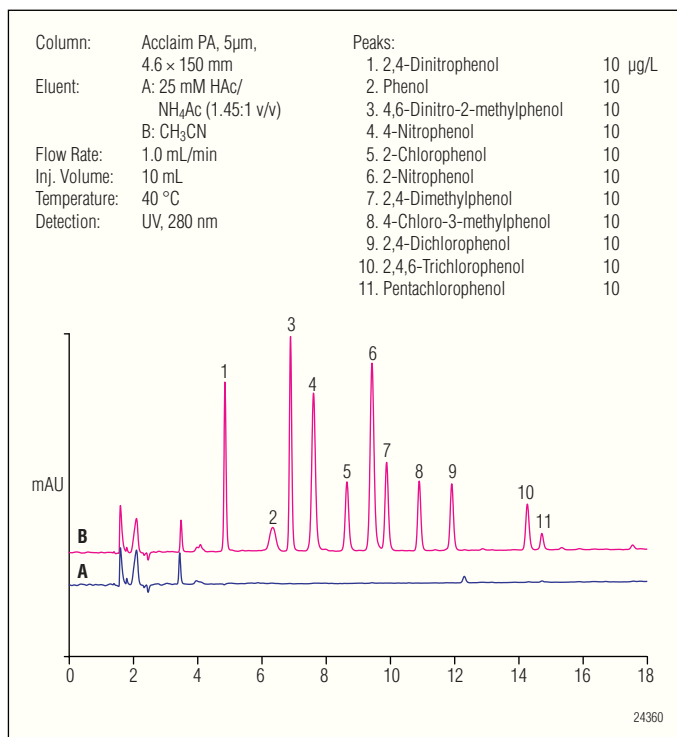


Figure 4. Overlay of chromatograms of bottled mineral drinking water 1, A) unspiked, and B) spiked with 10 µg/L phenols.

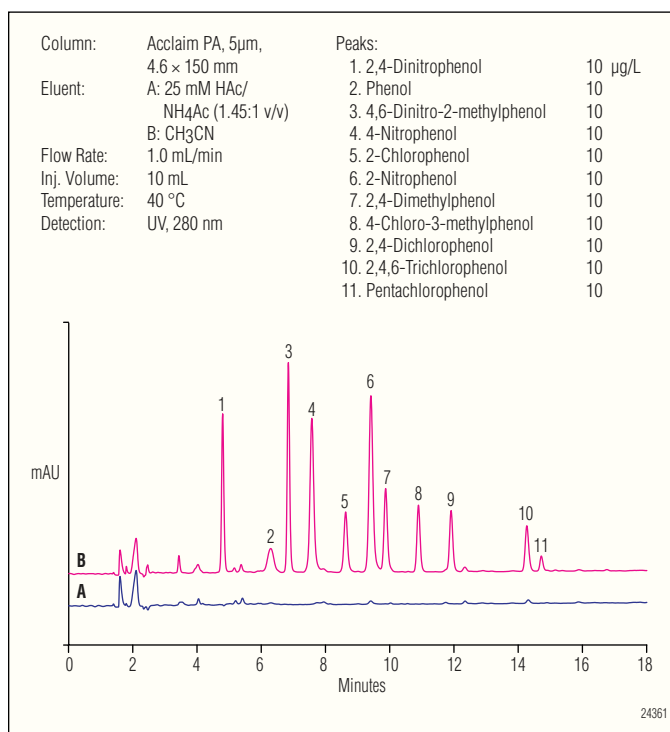


Figure 5. Overlay of chromatograms of bottled mineral drinking water 2, A) unspiked, and B) spiked with 10 µg/L phenols.

Table 6. Bottled Mineral Drinking Water Analytical Results								
Phenol	Bottled mineral drinking water 1 ^a				Bottled mineral drinking water 2 ^b			
	Unspiked (µM)	Added (µM)	Found (µM)	Recovery (%)	Unspiked (µM)	Added (µM)	Found (µM)	Recovery (%)
2,4-Dinitrophenol	ND ^c	10	9.44	94.4	ND	10	9.57	95.7
Phenol	ND	10	11.9	119	0.37	10	10.0	100
4,6-Dinitro-2-methylphenol	ND	10	9.56	95.6	ND	10	9.57	95.7
4-Nitrophenol	ND	10	10.2	102	ND	10	10.0	100
2-Chlorophenol	ND	10	10.4	104	ND	10	9.02	90.2
2-Nitrophenol	ND	10	11.9	119	ND	10	10.9	109
2,4-Dimethylphenol	ND	10	10.5	105	ND	10	9.97	99.7
4-Chloro-3-methylphenol	ND	10	9.56	95.6	ND	10	9.40	94.0
2,4-Dichlorophenol	ND	10	9.75	97.5	ND	10	9.05	90.5
2,4,6-Trichlorophenol	ND	10	10.1	101	0.75	10	9.55	95.5
Pentachlorophenol	0.73	10	9.67	96.7	ND	10	9.60	96.0

CONCLUSION

The successful analysis of all the water samples above demonstrates that online SPE with a dual UltiMate system can determine the 11 phenols designated on the EPA Priority Pollutants List without laborious offline sample preparation. The online SPE method with UV detection has very good reproducibility, with detection limits similar to and in many cases superior to the GC methods described in EPA Method 604.

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94088-3603
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