The On-line SPE-HPLC System

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Preconcentration and Detection of Herbicides in Water by Using the On-line SPE-HPLC System and Photochemical Reaction

Seung-Ho Lee,[†] Sung Kwang Lee, Young Hun Park, Hyunjoo Kim,[‡] and Dai Woon Lee*

Department of Chemistry, Yonsei University, Seoul 120-749, Korea
[†]*Research and Development Center, Korea Gas Corporation, Ansan 425-150, Korea*
[‡]*Division of General Education, Chung Ju National University, Chung Ju 380-702, Korea*
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The analysis of trace herbicides using the on-line SPE-HPLC system and a photochemical reaction was studied. 18 compounds of herbicides including eight triazines, six phenoxy acids and esters, and four other herbicides were examined. The on-line SPE-HPLC system developed for selection of eluting solvent improved chromatographic efficiency. The recoveries of herbicides were higher than 77%. With 100 mL tap water samples, the detection limits for all analytes were in the 0.1-2.3×10^{-10} M range. Detection was done by a UV or fluorescence spectrometer after photochemical reaction at the end of the column with 2W or 450W mercury lamp. Without a photochemical reaction, all compounds responded to 230 nm UV detector, but phenoxy acids and esters were weakly detected. However, with a photochemical reaction, these compounds were selectively detected at 320 nm wavelength of UV absorption and 400 nm emission of the fluorescence detectors. This method can be used for the analysis of environmental water containing herbicides at trace levels.

Introduction

Herbicides have been widely used for the crop cultivation, and their consumption has been growing. The herbicides sprayed on crops contaminate water and soil. Some of these are dissociated to poisonous materials such as phenolic metabolites that increase the severity of contamination.[1] Thus, it is of great importance to develop methods of separating herbicides for the analysis of water samples.

Triazines and phenoxy acids, which were used in this study, are herbicides widely used around the world. They are relatively stable and their residual time is very long.[2-4]

HPLC has been shown to be an effective technique for the determining of nonvolatile and thermolabile organic compounds. But trace analytes in a sample matrix generally do not permit direct detection by HPLC. Therefore, on-line sample preconcentration using solid phase extraction (SPE) and HPLC is necessary.

The On-line SPE-HPLC system allows easy automation at a relatively low cost, with high sample throughput and good reproducibility. However, the low flexibility in setting the desorption conditions of the on-line system is the problem that has to be coped with when one tries to obtain a narrow elution profile from the precolumn. Rodiers[5] *et al.* have reported that solvent focusing and elution technique using two switching valves improved chromatographic efficiency for the on-line SPE-HPLC system. They successfully employed the focusing technique in the sampling, derivatization, and analysis of atmospheric aldehydes and ketones as their dansylhydrazones.[6] They used C_{18} sorbent to minimize interferences due to ozone and water vapor. Therefore, we designed the on-line SPE-HPLC system, which adopts merits of Rodiers' on-line system.

Among the herbicides used in this study, phenoxy acids and esters are known to have poor sensitivity to a UV detector and can be interfered with sample matrices.

Recently, photolysis has been used as a LC postcolumn reaction for the sensitive determination of herbicides with a variety of detectors. de Kok *et al.*[7] preconcentrated N-methylcarbamate and metabolites in surface water by the off-line SPE method and determined these herbicides by post-column derivatization and fluorescence detection methods. Miles *et al.*[8] reported the application of more than 100 pesticides to fluorescence, electrochemical, and conductivity detectors after post-column photolysis. It was reported that sulfur-containing pesticides responded to an electrochemical detector, and nitrogenous pesticides responded to fluorescence detector. However, a conductivity detector did not show good sensitivity for multi-component analysis.

In this study, we focused on the on-line SPE-HPLC method and a photochemical derivatization reaction which have no interference effect from side products and can be easily automated and high reproducible.

The characteristics of analytes without and with photochemical reaction in each detector: UV, fluorescence, and conductivity detectors - were compared.

Experimental Section

Instrumentation. The HPLC system consisted of a LC-10AD pump and SPD-10A UV-VIS detector (Shimadzu, Kyoto, Japan), Rheodyne 7125 injector (Rheodyne, Inc., Cotati, CA, USA), two injection loops (20 and 50 μL), SLC-300 conductivity detector (Samsung Electronic, Suwon, Korea) and a S-3350 fluorescence detector (Soma optics,

Tokyo, Japan) and Chromatopac CR-6A integrator (Shimadzu, Kyoto, Japan). A three-way inlet valve (Hamilton, Reno, NV, USA), a ten-port C10W valve (Valco, Houston, TX, USA), a six-port stream selection V240 valve (Upchurch, Oak Harbor, WA, USA), and a six-port Rheodyne 7000 valve (Rheodyne, Inc., Cotati, CA, USA) were used for the on-line system (Figure 2). On-line sample loading was performed with MasterFlex Microprocessor pump (Cole-Parmer, Chicago, IL, USA). The precolumn (20×2 mm i.d.) was filled manually with packing material. A photochemical reactor was connected to the column outlet. Lamps used in photochemical reaction were 2W ozone-free low pressure mercury lamp (BHK Inc., POMONA, CA, USA, Model 80-1178-01, 180 mm $\times$ 9.0 mm o.d.) and 450W High-Pressure Quartz Mercury-Vapor Lamp (Hanovia Inc., Lehigh Avenue Union, NJ, USA, Cat. No. 7825-34, 129 mm). In the 450W mercury lamp, a UV power supply (Ace Glass Inc., Vineland, NJ, USA, Cat. No. 7830) was used. This experiment was conducted by fixing a PTFE (polytetrafluoroethylene) coil (7 m $\times$ 0.5 mm i.d.) around the lamp (Figure 3). The lamp was cooled by a cooling fan or water circuit to reduce the heat produced. LC-grade water was obtained from the Milli-Q system (Millipore, Bedford, MA, USA).

Reagents and standards. Chemical structures of the herbicides used in this study are represented in Figure 1. Ametryn and terbutryn were the products of Supelco (Bellefonte, PA, USA), dimethametryn (99.6%), and terbutylazine (96.5%) synthesized by agricultural company obtained from the Korea Basic Science Institute (Taejon, Korea). All other herbicides-2,4-dichlorophenoxyacetic acid (2,4-D), simazine, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), prometon, atrazine, propanil, propazine, 4-(2,4-dichlorophenoxy)-butyric acid (2,4-DB), terbutryn, methyl 2,4-dichlorophenoxyacetate (2,4-DME), isopropyl N(-3-chlorophenyl) carbamate (CIPC), dimethametryn, metolachlor, methyl 2,4,5-trichlorophenoxyacetate (2,4,5-TME), methyl 4-(2,4-dichlorophenoxy)-butyroate (2,4-DBME) - were purchased from Polyscience (Division of Preston Industries, Inc. Niles, IL, USA). The individual stock standard solutions of all herbicides were prepared in HPLC grade aqueous acetonitrile mixtures. The solid sorbents XAD-2 and XAD-4 were macroreticular resins purchased from Rohm & Haas (Philadelphia, PA, USA) and prepared by grinding and sieving (150-200 mesh). Soxhlet extractor using methanol and acetonitrile purified the resins.

Mobile phase and stationary phase. Mixtures of phosphate buffer (0.05 NaH$_2$PO$_4$) and acetonitrile (Burdick & Jackson Laboratories, Inc., Muskegon, MI, USA) were used as mobile phase (46 : 54, v/v): pH 4.3 was adjusted using 99.9% H$_3$PO$_4$. The mobile phase used was degassed by vacuum filtration through a 0.45 m nylon 66 filter (Alltech Associates, Deerfield, IL, USA) followed by agitation in ultrasonic bath. The flow rate of mobile phase was 1.0 mL/min. Supheriorex ODS column (5 μm, 250×4.6 mm i.d. Shiseido Co. Ltd., Tokyo, Japan) were used. The column was thermostatted at 40 °C using a home-made water bath.

Procedure. The on-line injection was performed by using two precolumns, four switching valves, and an injector. The
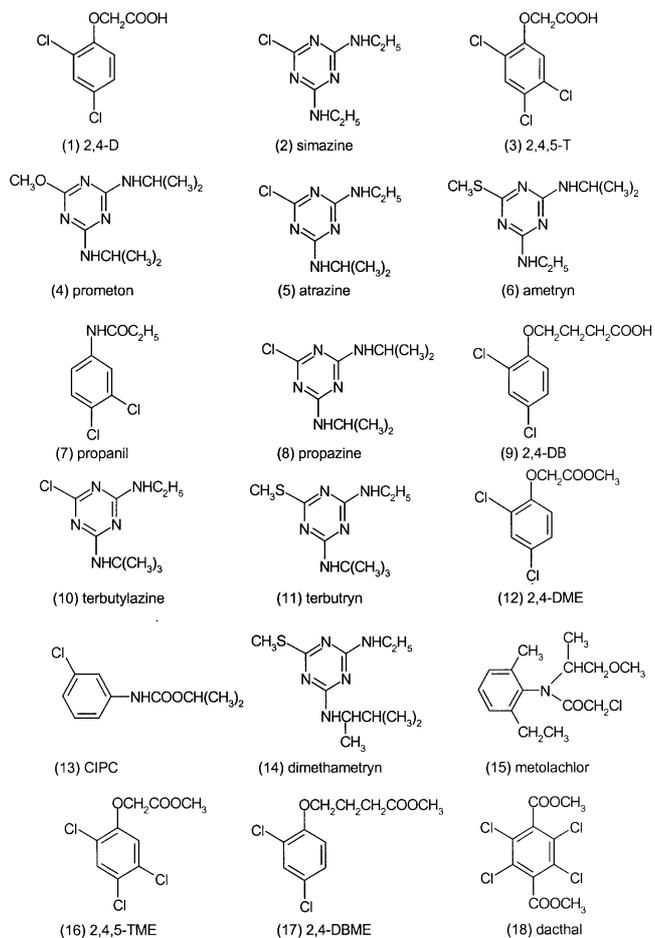
Figure 1. Chemical structures of 18 herbicides.

steps of preconcentration procedure are schematically represented in Figure 1 and summarized in Table 1. In a photochemical reactor analytes was irradiated with a high or low UV power source after analytes were eluted in an optimally separated condition. According to the power of light source, the experiment was performed with a different detection system (Figure 2). With low power lamp (2W), the lamplight can directly reach on reaction coil. The distance between the lamp and the reaction coil was 3cm. The surroundings were sealed with a high reflective efficiency stainless-steel case. With high power lamp (450W), due to the cooling device, the lamplight would not directly reach on the reaction coil. Therefore, the coil was fixed on a cooling condenser and wrapped with aluminum foil. Characteristic of analytes were studied, giving significantly different UV spectra (230 and 320 nm) with and without UV irradiation. With a fluorescence detector, the selectivity of the analytes was investigated at exciting and emitting wavelength. In each case, in order to study photochemical reaction efficiency, the results were compared with and without UV irradiation.

Results and Discussion

Preconcentration. In order to apply solid phase extraction to the on-line system, it is important to select sorbent

Table 1. The Procedure of Preconcentration Using Dual Injection On-line SPE-HPLC System

Step	Operation	pump control	path A	V1	V2	V3	V4	injector
						Valve position		
1	Washing the precolumn 1 (20 mm × 2 mm i.d., dry-packed) with 10 mL of ACN. (2.5 mL/min)	path B	-	ACN	1	load A	load B	load A
2	Conditioning them with 10 mL of MeOH and then 10mL of LC-grade water. (2.5 mL/min)	path B	-	MeOH H_2O	1	load A	load B	load A
3	Preconcentration of sample (5 mL/min)	path B	-	Sample	1	load A	load B	load A
4	Straightflush washing of precolumn 1 (path B) with 2.5 mL of water (2.5 mL/min)	path B	-	H_2O	1	load A	load B	load A
5	Backflush washing and filling of precolumn 1 (path A) and washing of precolumn 2 (path B) with 2.5 mL of water (2.5 mL/min)	path A and B	H_2O	H_2O	2	load B	load B	load A
6	Dual injection of desorption solution and precolum 1 to the analytical column. Washing of precolumn 2 and sample loading	path B	-	H_2O ACN MeOH H_2O sample	2	load B	load A	load B
7	Straightflush washing of precolumn 2 (path B), washing of precolumn 1 with 2.5 mL of water (2.5 mL/min) and washing injector.	path A and B	H_2O	H_2O	2	load B	load B	load A
8	Backflush washing and filling of precolumn 2 (path A) and washing of precolumn 1 (path B) with 2.5 mL of water (2.5 mL/min)	path A and B	H_2O	H_2O	1	load A	load B	load A
9	Dual injection of desorption solution and precolum 2 to the analytical column. Washing of precolumn 2 and repeat 19 steps.	path B	-	H_2O	1	load A	load A	load B
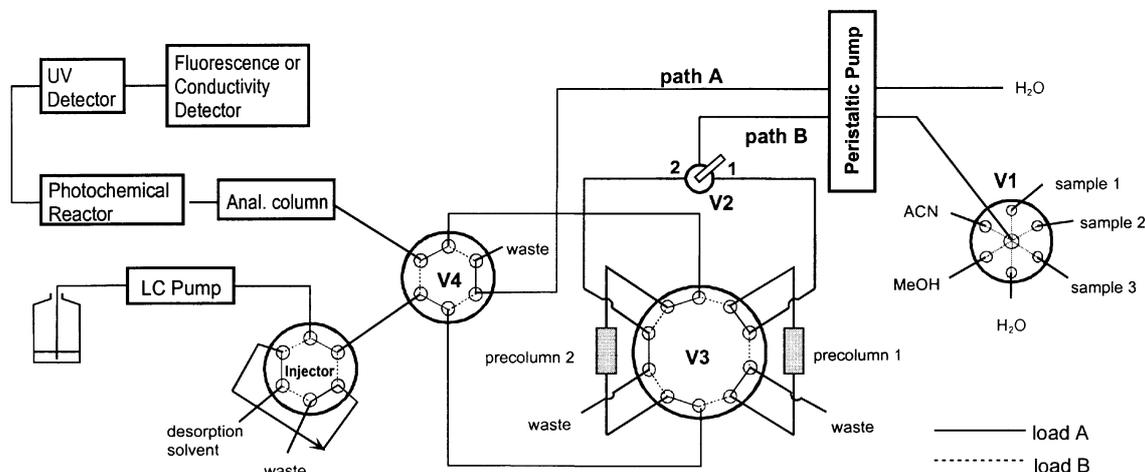
Figure 2. The schematic diagram of dual injection on-line SPE-HPLC system.

material, which shows high recovery values and no band broadening for herbicides. In the previous study,[9] XAD-2 and XAD-4 were found to be the most effective sorbents for triazines, as XAD-4 was for phenoxy acids and esters. Therefore, we applied XAD-4 to the on-line system because all herbicides were analyzed with high recovery values. Herbicides applied in this study are very sensitive to pH (Triazines: weak base, phenoxy acids: weak acid, phenoxy ester and other herbicides: neutral). For efficient adsorption, it is necessary to adjust the pH of the sample solution with trifluoroacetic acid (TFA). Varying the buffer pH from 3.0 to 4.5 affected recoveries of herbicides as shown in Table 2.

The recovery values were obtained by the peak areas corresponding to the off-line SPE extracts of a 100 mL water sample containing 4×10^{-9} M of triazines and 16×10^{-9} M

of other herbicides.

Since the number of interfering components in the chromatogram increases as pH decreases, the optimum pH was 3.5, and recovery values ranged from 81% to 102%. When phosphoric acid was used instead of TFA, the more interfering components were found.

On-line SPE-HPLC system. Generally, with the on-line SPE-HPLC method total analytes adsorbed were directly transferred from the SPE precolumn to the analytical column after the position of the switching valve was changed. The sorbent material in the SPE precolumn should be identical to the packing material in the analytical column. If two different sorbents are used, the retention of the analyte in the precolumn should be shorter than that in the analytical column.[10] However, it becomes more critical in the preconcentration of

Table 2. Effect of pH on the Recoveries (%) of Herbicides Spiked in 100 mL Water Sample on XAD-4 Adsorbent

No.	Compound	Recovery (%)			
		pH 3.0	pH 3.5	pH 4.0	pH 4.5
1	2,4-D	89 ± 5	92 ± 5	93 ± 3	61 ± 13
2	simazine	82 ± 4	99 ± 10	93 ± 7	106 ± 18
3	2,4,5-T	86 ± 5	91 ± 4	95 ± 3	69 ± 7
4	prometon	84 ± 5	90 ± 4	90 ± 9	89 ± 5
5	atrazine	93 ± 4	10 ± 24	109 ± 4	97 ± 4
6	ametryn	92 ± 5	96 ± 4	97 ± 3	95 ± 3
7	propanil	89 ± 3	90 ± 1	75 ± 2	79 ± 4
8	propazine	87 ± 4	91 ± 2	83 ± 3	89 ± 4
9	2,4-DB	86 ± 4	90 ± 1	83 ± 1	83 ± 1
10	terbutylazine	91 ± 3	95 ± 2	88 ± 1	83 ± 3
11	terbutryn	88 ± 4	93 ± 1	88 ± 2	86 ± 2
12	2,4-DME	87 ± 4	90 ± 1	83 ± 3	84 ± 3
13	CIPC	92 ± 4	94 ± 1	90 ± 1	89 ± 3
14	dimethametryn	91 ± 4	93 ± 1	88 ± 2	85 ± 2
15	metolachlor	83 ± 3	86 ± 3	83 ± 2	79 ± 3
16	2,4,5-TME	92 ± 6	93 ± 1	90 ± 2	88 ± 3
17	2,4-DBME	84 ± 4	86 ± 1	75 ± 4	77 ± 3
18	dacthal	80 ± 4	81 ± 1	71 ± 3	74 ± 4
	Average	88	92	87	84

Each value is the average of three replicates.

polar herbicides when a sorbent having stronger interactions with these analytes than conventional C_{18} material is used in the SPE precolumn. If the SPE precolumn containing more hydrophobic material (e.g. XAD-2 or XAD-4) is connected to a C_{18} analytical column, additional band broadening is obvious. Therefore, we designed the on-line SPE-HPLC system to solve the problem of band broadening. First, it permits the adjustment of eluting solvent by using a dual switching valve. Desorption is performed by a desorption solvent of an injector and is started by dual injection of both an injector and a switching valve 4 (Figure 2). The resulting narrow elution profile of the precolumn improves chromatographic efficiency. Second, it permits the continuous processing of two samples by using two precolumns. Third, it enables backflush washing to prevent the clogging of the precolumn before an on-line injection.

Figure 4 illustrates chromatographic efficiency results. Chromatogram A depict elution of herbicides obtained under the conditions of mobile phase desorption and B under conditions of THF desorption. All chromatogram preconcentrated 100 mL water sample that was spiked with 4×10^{-9} mol/L of triazines and 16×10^{-9} mol/L of phenoxy acids and others. Diverse solvents (ACN, THF, MeOH, hexane, and chloroform: 50 μL) were used for desorption. THF desorption method provided higher chromatographic efficiency than other solvents. The recovery values were obtained by comparing the peak areas corresponding to the extracts with those obtained by direct injection (20 μL).

The system was validated for 100 mL Milli-Q water sample. The reproducibility of peak areas and the capacity factors (k') were measured (Table 3). Coefficients of variation
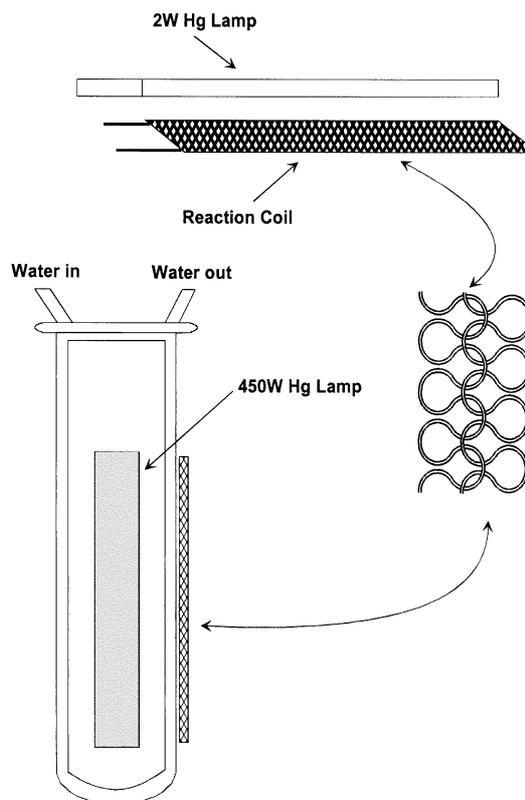
Figure 3. Diagram of photochemical reactor used in this study.
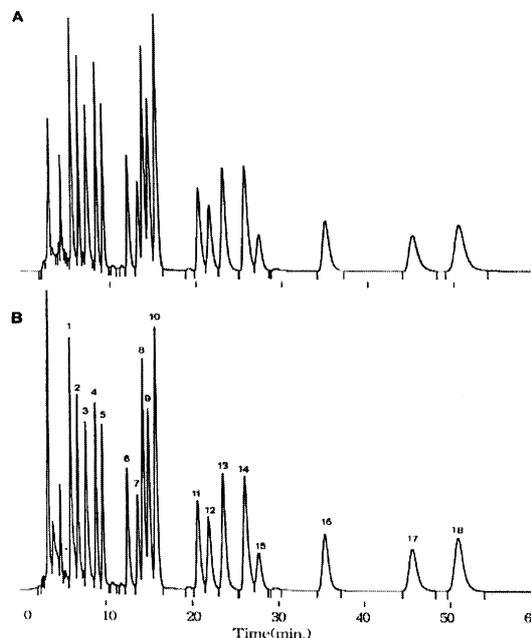
Figure 4. Chromatograms of 100 mL Milli-Q purified water sample spiked with herbicides. Eluting solvent: (A) mobile phase (B) 50% THF.

(CV) with on-line injection were in the range of 0.8-2.6%. The k' and CV of direct injection are almost the same as those of on-line injection.

The standard analytes were added to 100mL tap water and calibration curves were obtained. The concentrations of standard samples were 0.4, 1, 2, 3, 4×10^{-9} mol/L for triaz-

Table 3. Comparison between Direct and On-line Injections of Herbicides Spiked in 100mL Milli-Q Water Sample

No.	Compound	Direct injection		On-line injection	
		CV[a]	k'	CV[a]	k'
1	2,4-D	0.7	5.7	1.4	5.5
2	simazine	0.7	6.6	1.2	6.4
3	2,4,5-T	0.8	7.5	1.4	7.2
4	prometon	0.5	8.7	1.4	8.5
5	atrazine	0.5	9.4	0.9	9.2
6	ametryn	0.7	12.4	1.0	12.1
7	propanil	1.1	13.6	0.9	13.3
8	propazine	1.4	14.2	0.9	13.9
9	2,4-DB	1.3	14.8	1.3	14.6
10	terbutylazine	1.3	15.7	0.8	15.4
11	terbutryn	1.3	20.7	1.0	20.4
12	2,4-DME	1.1	22.0	0.9	21.7
13	CIPC	0.5	23.6	1.0	23.3
14	dimethametryn	1.1	26.2	1.1	25.8
15	metolachlor	2.4	27.8	2.6	27.5
16	2,4,5-TME	1.0	35.7	1.1	35.4
17	2,4-DBME	0.4	46.0	1.1	45.6
18	dacthal	1.9	51.6	1.8	51.1
	Average	1.0		1.2	

[a]Coefficient of variation (CV): standard deviation/mean $\times$ 100. Each value is the average of three replicates.

Table 4. Recoveries and Calibration Data of Herbicides Spiked in Tap Water

No.	Compound	Recovery (%)	R^2	Method Detection Limits[a] ($\times 10^{-10}$ M)
1	2,4-D	94	0.9995	1.1
2	simazine	88	0.9776	0.3
3	2,4,5-T	103	0.9966	0.9
4	prometon	101	0.9998	0.4
5	atrazine	101	0.9998	0.2
6	ametryn	99	0.9998	0.1
7	propanil	79	0.9990	2.3
8	propazine	86	0.9992	0.3
9	2,4-DB	79	0.9989	1.4
10	terbutylazine	92	0.9998	0.3
11	terbutryn	90	0.9995	0.3
12	2,4-DME	86	0.9994	1.1
13	CIPC	90	0.9999	0.9
14	dimethametryn	90	0.9996	0.2
15	metolachlor	83	0.9993	2.3
16	2,4,5-TME	89	0.9999	0.9
17	2,4-DBME	80	0.9998	1.0
18	dacthal	77	0.9992	0.8
	Average	89		

[a]S/N = 3. Each value is the average of three replicates.

ines and 1.6, 4, 8, 12, 16×10^{-9} mol/L for phenoxy acids and others. The recoveries of analytes were 77-103% and the average was 89% (Table 4). With 100 mL tap water samples, the detection limit of the on-line SPE-HPLC method (signal-to-noise ratio = 3) was in the $0.1-2.3 \times 10^{-10}$ M range for all analytes. The correlation coefficients were all satisfactory ($R^2 > 0.99$), except for simazine in the tap water. It means that correct quantitative results of an on-line preconcentration of non-spiked tap water sample can be obtained by calibration curves.

Efficiency of a photochemical reaction coil. Most of photochemical reactors employed quarts or Teflon tubing as reaction coils. Attempts to use quartz as reaction coils were unsuccessful because quartz are expensive and fragile and tight connection were not easily made. From the studies on the photochemical reactors, it was known that a knitted pattern coil minimized band-broadening more than any other shape.[11] In this study, a knitted reaction coil was made with Teflon. The dead volume of the reaction coil was 1.5 mL. Practically, as a photochemical reactor is connected to the end of a column and is detected by a UV detector, the retention time is increased due to the dead volume. However, it was observed that band broadening did not occur.

UV detector. For the characterization of analytes in a photochemical reaction, detection at various UV wavelengths was investigated. With a 2W low-power lamp, UV intensity of analytes was not influenced significantly by UV irradiation. With a 450W high-power mercury lamp, UV intensity of analytes was reduced about 50%. This means that the relative abundance of photolysis products increase from 2W to 450W lamp. Because the lamp power source is

not strong enough to photolize the analytes and the 2W lamp mainly emits a 254 nm line, the maximum absorption wavelength of analytes does not correspond (Figure 5).

Intensity of photolysis products detected at UV 320 nm was comparable to that detected at 230 nm. Chromatograms obtained at 320 nm with and without UV irradiation display significant changes in the peak intensity (Figure 6).

Absorbances of propanil and CIPC were increased over 30 times at 320 nm after photochemical reaction. Absorbance of 10 analytes - 2,4-D, 2,4,5-T, propanil, 2,4-DB, 2,4-DME, CIPC, metolachlor, 2,4,5-TME, 2,4-DBME, and dacthal - was also increased at 320 nm (Table 5). Especially, phenoxy acids and esters, which showed weak absorbance at 230 nm were detected selectively at 320 nm after photochemical reactions.

For environmental samples, it is expected that the detection at longer wavelengths to be more effective because the interference of matrix is decreased and the absorbance of mobile phase is reduced at a long wavelength.

Fluorescence detector. Analytes used in this study either did not respond or weakly responded to a fluorescence detector. Therefore, in optimally separated mobile phase, photochemical studies were performed by a fluorescence detector. With a 2W lamp, only the propanil responded to the fluorescence detector and some analytes showed a weak response. With a 450W lamp, eight analytes - 2,4-D, 2,4,5-T, propanil, 2,4-DB, 2,4-DME, CIPC, metolachlor, and dacthal - were detected (excitation: 320 nm, emission: 400 nm). Particularly, propanil and CIPC were shown to be highly photosensitive signals, because structural modification by 450W UV irradiation allow measurements at higher wavelength

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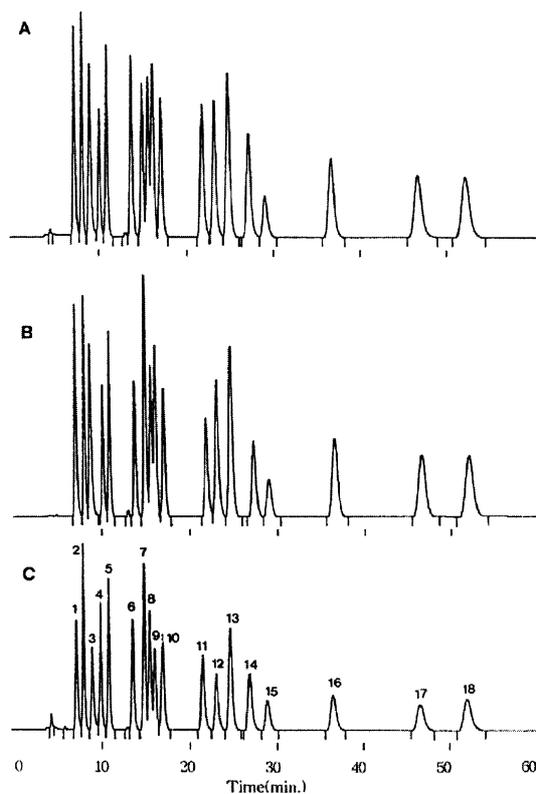
Figure 5. Effect of UV irradiation on response of analytes at UV 230 nm. (A) lamp off, (B) 2W lamp on, (C) 450W lamp on, AUFS = 0.128.
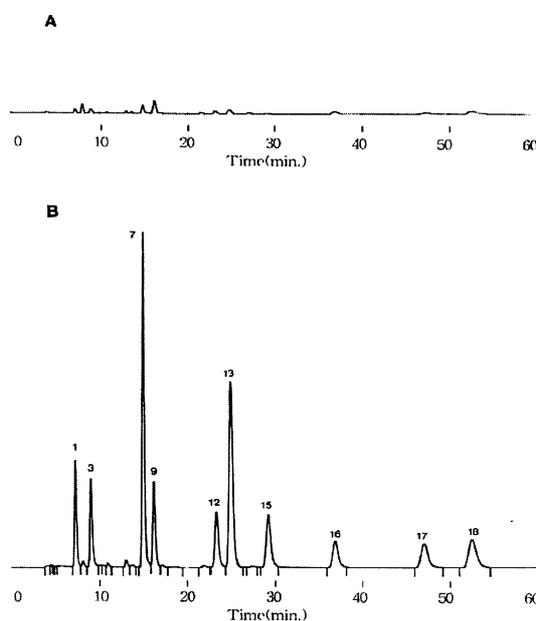
Figure 6. Effect of UV irradiation on response of analytes at UV 320 nm. (A) lamp off, (B) 450W lamp on, AUFS = 0.016.

Table 5. UV Characteristics of Herbicides with a Photochemical Reactor Lamp Off versus Lamp On

No.	Compound	Peak Area						
		230 nm			254 nm		320 nm	
		Lamp off	2W	450W	Lamp off	450W	Lamp off	450W
1	2,4-D	1637	1592	694	50	457	6	**100**
2	simazine	1544	1377	1233	193	387	11	5
3	2,4,5-T	1589	1468	610	136	390	8	**101**
4	prometon	963	935	925	166	162	2	2
5	atrazine	1500	1344	1148	170	395	3	4
6	ametryn	1650	1183	970	478	305	4	3
7	propanil	1157	1891	1029	3963	877	11	**384**
8	propazine	853	742	751	NR	235	NR	NR
9	2,4-DB	1075	1011	416	17	279	19	**104**
10	terbutylazine	1347	1224	937	158	398	2	2
11	terbutryn	1660	1173	948	469	334	4	3
12	2,4-DME	1780	1711	637	40	392	6	**93**
13	CIPC	2294	2311	1178	693	829	10	**331**
14	dimethametryn	1590	1145	875	454	279	4	3
15	metolachlor	723	640	517	82	365	3	**123**
16	2,4,5-TME	1634	1594	629	97	338	8	73
17	2,4-DBME	1664	1611	601	46	356	6	**87**
18	dacthal	1817	1763	876	120	500	14	**118**

NR = not respond. Each value is the average of three replicates.

Table 6. Detection Limit of Herbicides after a Photochemical Reaction

No.	Compound	Limit of Detection[a] (ng)			
		Fluorescence		Conductivity	
		2W	450W	2W	450W
1	2,4-D	NR	30	NR	NR
2	simazine	NR	NR	7.4	6.1
3	2,4,5-T	NR	77	8.4	4.8
4	prometon	NR	NR	NR	NR
5	atrazine	NR	NR	4.8	NR
6	ametryn	NR	NR	17	NR
7	propanil	14	9.5	2.1	0.9
8	propazine	NR	NR	12	NR
9	2,4-DB	NR	124	NR	NR
10	terbutylazine	NR	NR	4.8	1.2
11	terbutryn	NR	NR	10	NR
12	2,4-DME	NR	140	NR	NR
13	CIPC	188	8.7	4.6	0.97
14	dimethametryn	NR	NR	8.0	2.0
15	metolachlor	346	41	2.8	2.2
16	2,4,5-TME	NR	NR	13	1.2
17	2,4-DBME	NR	NR	NR	2.2
18	dacthal	476	133	7.7	1.8

NR = not respond. [a]S/N=3. Each value is the average of three replicates.

(320 nm) to be carry out. This was similar to the result obtained at UV 320 nm (Table 5, 6 and Figure 7).

Conductivity detector. Because the conductivity of the mobile phase for optimal separation was high (1200 μS/cm), analytes could not be detected at conductivity detector. The conductivity of the mobile phase mixed with pure water and acetonitrile (54/46 = v/v) was about 1-2 μS/cm. After adjusting pH of the mobile phase to 4.3 with phosphoric acid for separation, its conductivity was relatively low at 5 μS/cm. To keep pH conditions, the mobile phase was adjusted at pH 4.3 with 1 mM NaH_2PO_4. Background conductivity was
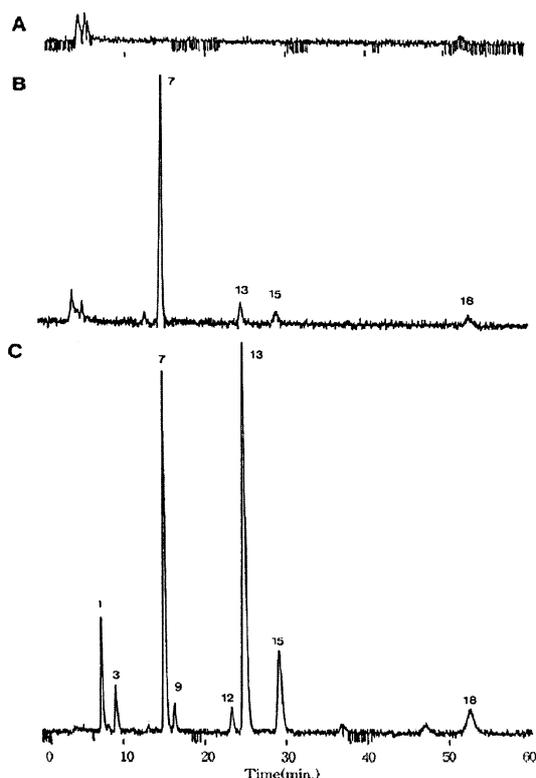
Figure 7. Effect of UV irradiation on response of analytes to fluorescence detector. (A) lamp off, (B) 2W lamp on, (C) 450W lamp on, λ_{ex} = 320 nm; λ_{em} = 400 nm, range = 8, attenuation = 5.

about 40-45 μS/cm, we could detect analytes. Since the composition of mobile phase was varied, elution order of some analytes was changed. After this experiment, we used an optimal mobile phase (1 mM NaH_2PO_4/ACN = 54/46, pH 4.3) for all experiments.

The 450W lamp was more effective than the 2W mercury lamp at photolysis-conductivity detection. Responses with the 450W lamp was increased to about two to ten times as much as that with the 2W lamp. Ten analytes were detected. Propanil, terbutylazine, CIPC, 2,4,5-TME, and dacthal were relatively sensitive, and simazine, 2,4,5-T, dimethametryn, metolachlor, and 2,4-DBME were detected. However, 2,4-D, prometon, atrazine, ametryn, propazine, 2,4-DB, terbutryn, and 2,4-DME were not detected. With the 450W lamp, a detection limit of some analytes by the conductivity detector was lower than that by the fluorescence detector (Table 6).

From the experiment of photochemical reaction efficiency with UV detector, we found that a photochemical reaction was not effective on an analyte that has a high molar absorptivity at a short wavelength, since the 2W low-pressure mercury lamp emits mainly a spectrum of 254 nm. The photochemical reaction with the 450W high-pressure mercury lamp allow measurements of some analytes at higher wavelengths (320 nm) to be performed. Most of these analytes responded to the fluorescence detector (ex. 320 nm, em. 400 nm). According to other research,[12] some pesticides that show higher fluorescence intensity after a photochemi-

cal reaction can produce phenolic moieties. Because an ionization of phenolic groups often results in more conjugation, the fluorescence of it in basic conditions increased and it absorbed a long wavelength. This research asserts that, except for propanil and the carbamate group, analytes did not respond to fluorescence. However, we could detect other analytes in our study. Because of this result, we knew that the radiation source must be selected according to the analytes.

Conclusion

The on-line SPE-HPLC system was developed for the monitoring of herbicides at trace levels (sub-ppb) in an aqueous sample. This system enables the changing of desorbing solvent to minimize additional band broadening in the analytical column and to use a series of precolumns containing different packing materials or a selective precolumn to enhance selectivity for diverse sample matrices. When we applied this system to tap water, we obtained high recoveries and reproducible data.

When detection was performed after post-column photochemical reactions, with a UV detector, we could detect analytes at long wavelengths where they were little interfered with a sample matrix. For photochemical reactions, a 450W high-pressure mercury lamp was more effective than a 2W low-pressure mercury lamp. Some analytes could be selectively detected on a fluorescence detector that otherwise could not detect them without a photochemical reaction. If the study on promoting photochemical reaction efficiency and carrying out simultaneous on-line SPE is done, we can expect that this detection will be more selective and sensitive.

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