Investigation of Retention Behavior for Herbicides in Micro-HPLC with Temperature Changes

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Investigation of Retention Behavior for Herbicides in Micro-HPLC with Temperature Changes

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ABSTRACT

The purpose of this study is to improve the detection limit for trace herbicides, and to separate all samples within a short time with the combination of temperature and flow programming with micro-reversed-phase liquid chromatography (RPLC). The optimum condition for separation was ACN : 0.05 M NaH_2PO_4 (46 : 54) (v/v), pH $= 4.3$, 40°C. The mass sensitivity is in the range between 0.086×10^{-13} g and 3.34×10^{-13} g in micro-HPLC (HPLC). Micro-HPLC exhibits higher mass sensitivity and better heat transfer than conventional HPLC. Thus, in the case of micro-HPLC, the retention behavior of herbicides is easily determined according to the change of temperature. Especially, retention of triazines

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3357

is decreased to a lesser extent with the increase of column temperature than that of other herbicides. This tendency has been accounted for the thermodynamic parameters ΔH° and ΔS°. The ΔS° for triazines is less negative than that for phenoxy acids, esters, and others. The chromatograms of herbicides were obtained within a short time with using both temperature and flow gradient, than with isocratic, isothermal, and isorheic conditions. When the mobile phase flow rate and the column temperature were changed simultaneously during the separation run, the analysis time was shortened up to 50%, while the separation efficiency was preserved.

INTRODUCTION

Many kinds of studies related to herbicides have been performed over the last few decades.[1–3] It is not surprising that herbicides considerably contribute to the contamination of soil, surface, and ground water, and particularly to the human body through the ecological food chain. For a long time, many nations have strictly set the tolerance limit for the contamination of environments for its effects on crops, water, soil, and so on. Therefore, it is of great importance to develop analytical methods for trace herbicides in environmental samples. Of the most popular techniques, high performance liquid chromatography (HPLC) is often preferred because its sample pretreatment is easy, there is no need for derivatization and no risk of thermal instability, and it is easy to operate.[4,5] Unfortunately, its sensitivity is often not sufficient to monitor analytes at low level concentrations. Therefore, in some cases, HPLC analysis requires a derivatization of analytes to improve detection sensitivity. Besides, it usually demands selective sample pretreatment methods,[6,7] followed by highly sensitive detection techniques to attain information on ultra-trace components in environmental pollutants. In previous works,[8] on-line SPE methods have been applied to herbicides and various detection modes were compared, including UV, fluorescence, and electrochemical and conductivity detection. Apart from the detection sensitivity, analysis time is also a very important factor. To meet the detection limit and analysis time simultaneously, temperature-programmed micro-HPLC was chosen in this study.

Micro-HPLC[9,10] is expected to be, due to its relatively low heat capacity and small diameter, more suitable for temperature programming than conventional HPLC. Usually, a temperature or solvent programming method is useful for improving resolution.[11] Especially, the former can be combined with a flow

programming method to reduce analysis time while maintaining resolution.[12] In the present study, the detection sensitivity of the conventional and microcolumn systems was compared using a UV detector. Also, the retention behavior of herbicides was investigated for a micro-HPLC condition, and retention trends were explained in terms of thermodynamic parameters.[13] Finally, the optimum separation condition was determined by a simultaneous controlling of the temperature and flow.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a LC-10AD pump (Shimadzu, Kyoto, Japan), a Linear UVIS 200 detector (Reno, NV, USA), and a SLC-500 column oven (Samsung Electron Devices, Suwon, Korea). The detector signal was processed using a PeakSimple (SRI instrument, Torrance, CA) program in an IBM PC compatible computer. Samples were injected using a Rheodyne model 7125 (20 µL) and 7120 (0.5 µL) loop injector (Rheodyne, Inc., Cotati, CA). Deionized water was purified in a Milli-Q system (Millipore, Bedford, MA) to obtain HPLC-grade water for use in mobile phase and sample solutions. For micro-HPLC, the flow rate was controlled by connecting the back pressure regulator located between the pump and injector. The split-flow technique was also used for the delivery of reproducible and stable micro-flows.

Mobile Phase and Stationary Phase
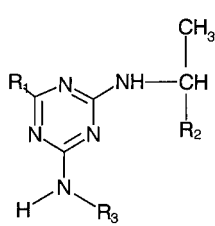
An acetonitrile/0.05 M NaH_2PO_4 buffer (46 : 54, v/v) was used as a mobile phase. The pH was adjusted to 4.3 by an addition of 99.9% H_3PO_4. The mobile phase was degassed by vacuum filtration through a 0.45 µm nylon 66 filter (Alltech Associates, Deerfield, IL), followed by agitation in an ultrasonic bath (Fisher Scientific Inc., Pittsburgh, PA). Supheriorex ODS (5 µm, 250 × 4.6 mm i.d. Shiseido Co. Ltd., Tokyo, Japan) was used as a conventional column and Capcell Pak ODS (5 µm, 250 × 1 mm i.d. Shiseido Co. Ltd., Tokyo, Japan) as a microbore column.

Chemicals and Reagents
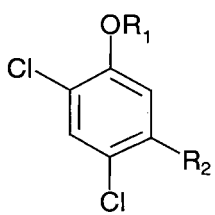
The chemical structures of the 18 herbicides used in this study are represented in Fig. 1. Ametryn and terbutryn were the products of Supelco (Bellefonte, PA) and dimethanmetryn (99.6%) and terbutylazine (96.5%) were

Triazines

	R_1	R_2	R_3
ametryn	CH_3S	CH_3	C_2H_5
atrazine	Cl	H	$(CH_3)_2CH$
dimethametryn	CH_3S	H	$(CH_3)_2CHCH(CH_3)$
prometon	CH_3O	CH_3	$(CH_3)_2CH$
propazine	Cl	CH_3	$(CH_3)_2CH$
simazine	Cl	H	C_2H_5
terbutryn	Ch_3S	H	$(CH_3)_3C$
terbutylazine	Cl	H	$(CH_3)_3C$

Phenoxyacetic acids and esters

	R_1	R_2
2, 4-D	CH_2COOH	
2, 4-DB	$CH_2 CH_2 CH_2COOH$	
2, 4, 5-T	CH_2COOH	Cl
2, 4-DME	CH_2COOCH_3	
2, 4-DBME	$CH_2 CH_2 CH_2COOCH_3$	
2, 4, 5-TME	CH_2COOCH_3	Cl

CIPC   dacthal

metholachlor   propanil
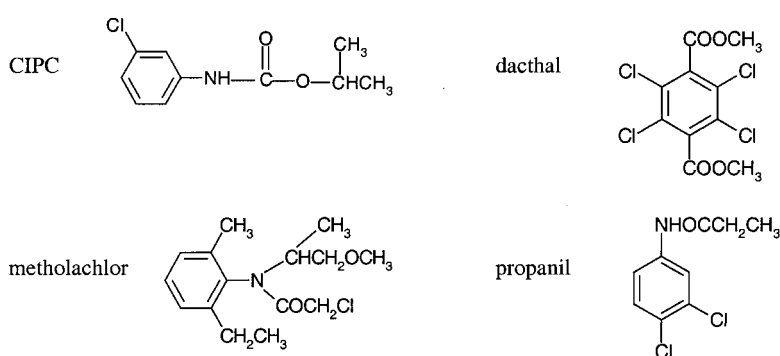
Figure 1. Chemical structures of 18 herbicides.

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obtained from the Korea Basic Science Institute (Daejon, Korea). Other herbicides were purchased from PolyScience (Division of Preston Industries, Niles, IL) corporation. High performance liquid chromatography-grade solvents were obtained from Burdick & Jackson Laboratories (Muskegon, MI). The individual stock solutions of all herbicides were prepared in HPLC-grade aqueous acetonitrile mixtures. Sample mixtures were injected into conventional (20 μL) and micro-HPLC (0.5 μL), respectively. All measurements were repeated at least three times and the average values were used.

Procedure

To investigate the detection limit in conventional and micro HPLC accurately, samples were divided into trizines, penoxyacetic acids, and esters, and the others. Each of them was experimented at five points. From the results, we acquired calibration curves as statistical method used by Long et al.[14] The signal to noise ration was used as 3. The retention time of herbicides was measured at the flowrate of 0.045 mL/min, varying the temperature, ranged from 27°C to 95°C. Temperature gradient was performed with the control of flow splitter after determining the temperature programming rate (r).

RESULTS AND DISCUSSION

First of all, the sensitivity and the detection limit of conventional HPLC were compared with those of micro-HPLC. The calculated down-scale factor was 21, obtained from the ratio of the diameter of a conventional column to that of a micro-column. This means that the latter is 21 times more sensitive than the former theoretically. Therefore, the detection limit in a micro-column is better than that in a conventional column. Calibration curves were obtained with 18 samples containing different concentrations, and good linearity was observed for all compounds ($R^2 \geq 0.99$). On the whole, the mass sensitivity of micro-HPLC is lower than that of conventional HPLC by as much as $7 \sim 14$ times (Table 1). Figure 2 shows the typical chromatogram of 18 herbicides at optimal separation condition. The separation of all compounds except (2) and (3) was achieved using an isothermal (313 K) and constant flow (45 μL/min) conditions.

Secondly, the retention behaviors of the 18 herbicides were investigated with temperature change in micro-HPLC. Micro-HPLC exhibits a higher mass sensitivity and better heat transfer than it does in conventional HPLC. Thus, in the case of micro-HPLC, the retention trends of herbicides could be easily characterized according to a change in temperature. Column efficiency generally increases with increasing temperature since diffusion coefficients,

Table 1. The detection limit for herbicides at conventional and micro HPLC.

No.	Solutes	Mass sensitivity (10^{-12} g)	
		Conventional HPLC	Micro-HPLC
1	2,4-D	0.188	0.0516
2	Simazine	0.0567	0.00860
3	2,4,5-T	0.132	0.0383
4	Prometon	0.0591	0.0181
5	Atrazine	0.0424	0.0137
6	Ametryn	0.0769	0.0218
7	Propanil	0.300	0.0914
8	Propazine	0.0703	0.0241
9	2,4-DB	0.278	0.0824
10	Terbutylazine	0.0861	0.0213
11	Terbutryn	0.187	0.0438
12	2,4-DME	0.366	0.105
13	CIPC	0.413	0.136
14	Dimethametryn	0.143	0.0673
15	Metolachlor	0.787	0.309
16	2,4,5-TME	0.541	0.203
17	2,4-DBME	0.813	0.334
18	Dacthal	0.665	0.323
R^2 range		$0.9988 \sim 1$	$0.9978 \sim 1$

which control the kinetics of mass transfer, increase with a rise of temperature. In Fig. 3, the chromatograms were compared under the different temperature conditions. As the temperature increased, the retention time was about three times faster at 368 K than it was at room temperature (300 K), for the viscosity of the eluent is decreased at a higher temperature. However, the separation efficiency was not satisfied at a high temperature condition and the elution order of some analytes was changed, such as simazine (2) and 2,4,5-T (3), propanil (7) and propazine (8), 2,4-DB (9) and terbutylazine (10), terbutryn (11), 2,4-DME (12) and CIPC (13) and dimethametryn (14) and metolachlor (15). The results were expressed with a van't Hoff plot in Fig. 4 (the dotted lines represent triazines).

It is interesting to note that the retention of triazine compounds was shown to be affected very little by temperature change. This is the reason why we investigated enthalpy and entropy with temperature change, which indicates the transfer of solutes from a mobile to a stationary phase. The enthalpy and entropy were calculated from the slope and intercept of a plot of *lnk'* vs. 1/*T*.
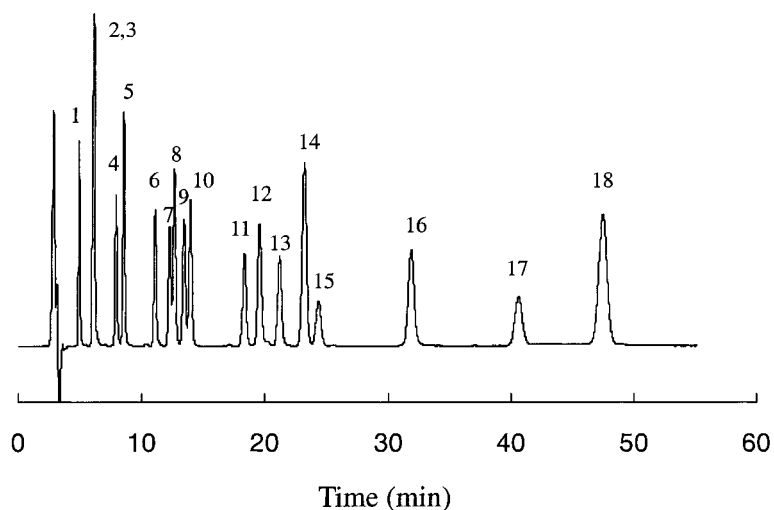
Figure 2. Chromatograms of 18 herbicides in micro-HPLC. Microcolumn (250×1.0 mm i.d.); Capcell Pak ODS particle size $5\,\mu$m; ACN/0.05 M NaH$_2$ PO$_4$ 46 : 54 (v/v), pH $= 4.3$, flow rate $45\,\mu$m/min.; column temperature 313 K; UV detection at 254 nm. Peaks (1) 2,4-D (2) simazine (3) 2,4,5-T (4) prometon (5) atrazine (6) ametryn (7) propanil (8) propazine (9) 2,4-DB (10) terbutylazine (11) terbutryn (12) 2,4-DME (13) CIPC (14) dimethametryn (15) metolachlor (16) 2,4,5-TME (17) 2,4-DBME (18) dacthal.

The phase ratio (φ), which is the volume ratio of stationary phase to mobile phase, was calculated by the method of Jandera et al.[15] As shown in Table 2, linear van't Hoff plots were observed in the investigated temperature range. The negative values of ΔH° and ΔS° for all solutes are typical of the reversed-phase liquid chromatography (RPLC) mechanism. Especially, the entropy change of triazine compounds was shown to be less negative than was that of the others. This result can be explained by relating the structure of analytes to that of acetonitrile, used for mobile phase. The carbon–nitrogen resonance structure of triazines has a weaker interaction with acetonitrile than the carbon–carbon resonance structure of phenoxy acids. As a result, the transfer of triazines from the mobile to the stationary phase is more favorable, and leads to a longer retention. To verify this proposition, a confirmatory analysis was performed employing various polycyclic aromatic hydrocarbons (PAHs) (data not shown).[16] The results show that, according to the increase in temperature, the retention decreased and its elution order also changed, especially due to the rapid decrease in nitro PAHs.
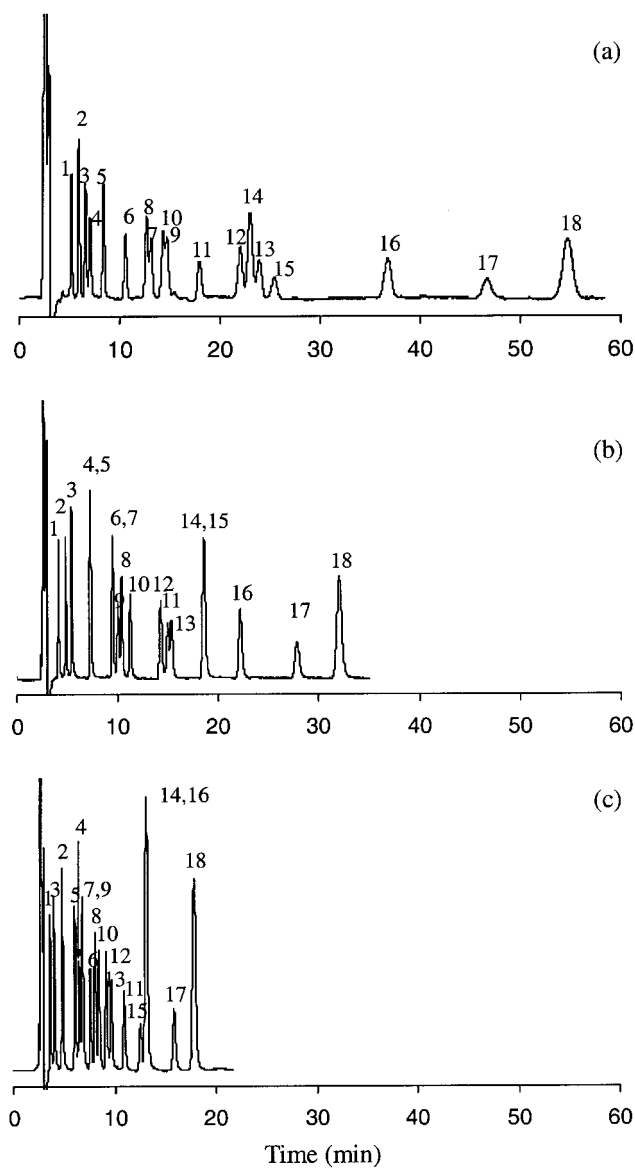
Figure 3. Chromatograms of 18 herbicides under different temperature conditions. (a) 300 K, (b) 328 K, and (c) 368 K. Other conditions and peak identification as in Fig. 2.

(1)2.4- D   **(2)simazine**
(3)2,4,5- T   **(4)prometon**
(5)atrazine   **(6)ametryn**
(7)propanil   **(8)propazine**
(9)2,4- DB   **(10)terbutylazine**
(11)terbutryn   **(12)2,4- DME**
(13)CIPC   **(14)dimethametryn**
(15)metolachlor   **(16)2,4,5- TME**
(17)2,4- DBME   **(18)dacthal**
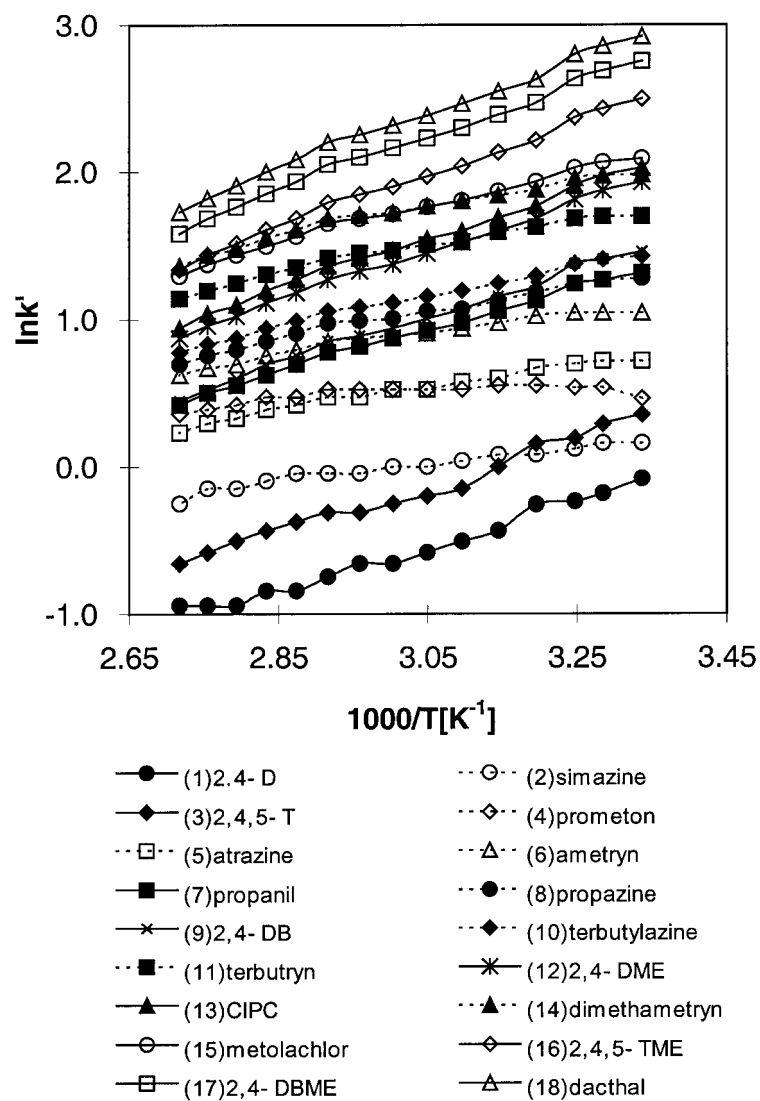
Figure 4. The van't Hoff plot of 18 herbicides.

Table 2. Thermodynamic data for herbicides under different temperature conditions.

No.	Solutes	R^{2a}	ΔH° (kcal/mol)	ΔS° (cal/mol·K)	ΔG°(313 K) (kcal/mol)	ΔG°(348 K) (kcal/mol)	ΔG°(368 K) (kcal/mol)
1	2,4-D	0.9741	−2.6756	−8.7391	0.0596	0.3655	0.5403
2	Simazine	0.9186	−1.2152	−3.1425	−0.2316	−0.1216	−0.0588
3	2,4,5-T	0.9585	−2.9209	−8.6662	−0.2084	0.0949	0.2683
4	Prometon	0.8742	−0.7450	−0.6981	−0.5266	−0.5022	−0.4882
5	Atrazine	0.9701	−1.6422	−3.3939	−0.5799	−0.4611	−0.3932
6	Ametryn	0.9716	−1.5793	−2.4649	−0.8078	−0.7215	−0.6722
7	Propanil	0.9924	−2.8533	−6.3253	−0.8735	−0.6521	−0.5256
8	Propazine	0.9737	−1.9064	−3.1942	−0.9066	−0.7948	−0.7309
9	2,4-DB	0.9894	−3.1414	−7.0376	−0.9386	−0.6923	−0.5516
10	Terbutylazine	0.9763	−2.1015	−3.5786	−0.9814	−0.8561	−0.7846
11	Terbutryn	0.9753	−1.9590	−2.4504	−1.1920	−1.1063	−1.0573
12	2,4-DME	0.9947	−3.2966	−6.6277	−1.2221	−0.9902	−0.8576
13	CIPC	0.9913	−3.3511	−6.6188	−1.2794	−1.0478	−0.9154
14	Dimethametryn	0.9638	−2.0653	−2.2637	−1.3568	−1.2775	−1.2323
15	Metolachlor	0.9812	−2.5300	−3.6734	−1.3802	−1.2517	−1.1782
16	2,4,5-TME	0.9910	−3.5269	−6.2933	−1.5571	−1.3368	−1.2110
17	2,4-DBME	0.9809	−3.5941	−5.9861	−1.7195	−1.5099	−1.3901
18	Dacthal	0.9909	−3.6752	−5.9269	−1.8201	−1.6126	−1.4941

[a]Value for the linear fit of the van't Hoff plot.

In the same way, the nitro group of nitro PAHs would be expected to have a stronger interaction with the nitrile group of acetonitrile, the main component of mobile phase. This makes the unfavorable transfer of solutes from a mobile to a stationary phase and this leads to a larger negative entropic value. The difference in entropic value between the PAHs and herbicides would be more pronounced, for the planarity of PAHs would be expected to have a more steric effect on a stationary phase. More detailed research on the retention behavior of PAHs with temperature is now in progress by means of quantitative structure-retention relationships (QSRRs) data. Regarding van't Hoff plots in Fig. 4 and R^2 values in Table 2, some of data were not so linear because we used very short temperature range to get more accurate data. This may cause inhomogenous retention ability of the stationary phase. However, this is not critical for explaining the retention trends described above.

The effect of temperature changes on selectivity was applied to separate the 18 herbicides within a short time. To achieve a simultaneous temperature and flow gradients, it was necessary to place the split capillary outside the column oven and to maintain it at a constant temperature. Figure 5 shows the separation of a mixture of 18 herbicides using simultaneous temperature and flow gradient. As shown in Fig. 5(a), although the run time was reduced to less than 20 min, some kinds of compounds [propanil (7) and propazine (8), dimethanetryn (14) and metoachlor (15)] were not separated. In this run, a temperature gradient was applied from 300 to 363 K with a heating rate (r) of 7.29 K/min (from 300 to 318 K) and 6.22 K/min (from 318 to 363 K), respectively. The initial flow rate was 45 μL/min and the final flow rate was changed to 90 μL/min. To separate all the compounds while maintaining separation efficiency, a multistep temperature change was carried out by manual handling. Initially, the temperature was elevated to 353 K ($r = 8.35$ K/min) and was then lowered to 318 K ($r = -13.01$ K/min) after a one minute hold. Finally, the temperature was elevated to 353 K again and maintained. The initial flow rate was 48 μL/min and the final flow rate was observed to be 112 μL/min. In this condition, all analytes were separated within 20 min.

Consequently, the improvement of the detection limit and separation were accomplished with micro-HPLC. When the flow rate and the column temperature were controlled simultaneously during the separation run, the analysis time was reduced up to 50%, while the separation efficiency was preserved.

CONCLUSION

First of all, the 18 herbicides were successfully separated within 30 min with high sensitivity in a micro-HPLC system. Secondly, the retention
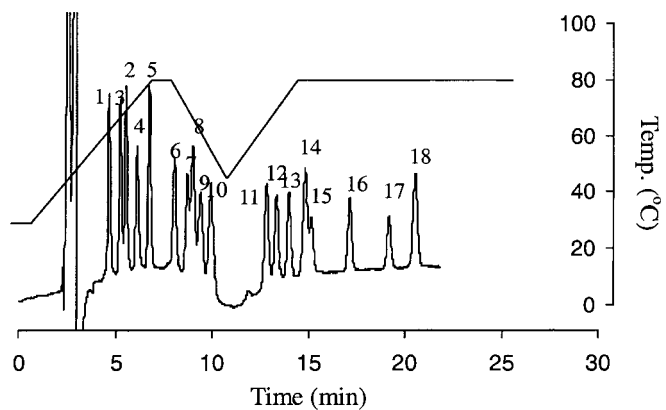
(a)
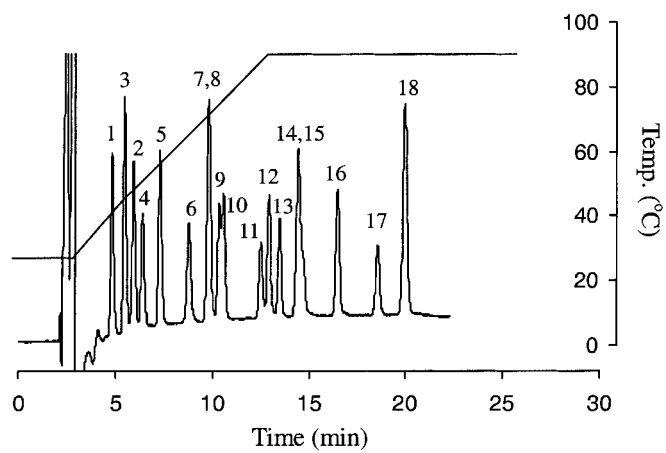
(b)

Figure 5. Separation of 18 herbicides with the combination of temperature and flow gradients.

behavior was reasonably explained according to the change of temperature. Especially, retention of triazines is decreased to a lesser extent with the increase of column temperature than that of other herbicides. This tendency has been accounted for in the thermodynamic parameters ΔH° and ΔS°. These results can be used as a good indicator for setting up the analyzing condition of these kinds of environmental compounds.

ACKNOWLEDGMENT

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