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Pesticide analysis by high-performance liquid chromatography using the direct injection method

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Abstract

HPLC with direct injection was examined as a simple and rapid method for the determination of pesticides in water. Fifteen pesticides listed in the Japanese standard and guidelines for potable water were separated successfully with an ODS column and acetonitrile–phosphate buffer as the mobile phase. All pesticides were separated simultaneously under gradient elution conditions: $[CH_3CN] = 30\%$ at 0 min to 80% at 60 min, flow-rate = 1.5 ml/min. However, isocratic elution conditions were required for large injection volumes. Even with a 5-ml injection, successful chromatograms were obtained and all pesticides were detected at the ppb or sub-ppb level. A 5-ml environmental water sample was also injected after filtration with a glass-fibre filter (0.3 μm) and propyamide, MEP and diazinon were observed at 0.07, 1.3, 1.1 μg/l, respectively.

Keywords: Environmental analysis; Water analysis; Pesticides

1. Introduction

Pollution of water with pesticides has become widespread and effects on human health via drinking water are of concern. Recently, fifteen pesticides were added to the standard and guidelines for potable water in Japan. These require both more extensive environmental monitoring and the development of water treatment technology for pesticide removal. For these reasons, it is necessary to develop much simpler procedures for pesticide determination.

In common procedures for water analysis, pesticides are separated from the aqueous matrix before chromatographic measurements. In general, liquid–liquid and solid-phase extraction are adopted. However, these techniques are time consuming, require high-purity solvents and are expensive. On-line solid-phase extraction techniques using precolumns [1–10] are excellent methods for rapid analysis. Solid-phase extraction has been carried out not only with a single adsorbent but also with multiple adsorbents [6–8] in order to recover a wide range of pollutants, e.g., C_{18}, styrene–divinylbenzene polymer, porous graphitic carbon and cation-exchange resins. Membrane extraction discs were also introduced for the on-line solid-phase extraction of pesticides in aqueous samples [11]. In a column-switching method, direct injection was also examined [9,10] for rapid analysis. In any case,

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however, a high recovery is required for these extraction processes.

A direct injection method without column switching has also been examined in HPLC measurements [12,13]. This method may provide an alternative on-line solid phase extraction to the column switching method and in this method there is no need to consider the recovery attendant on the extraction for pretreatment of an aqueous sample. In conventional pesticide analysis, HPLC is generally limited to a few pesticides such as thermally labile compounds and electrolytes. However, when the separation efficiency for a wide range of pesticides has been clarified and the detection level may be improved, HPLC with direct injection should be of use as an alternative procedure.

In this work, the pesticides included in the Japanese standard and guidelines for potable water were used as samples and the factors influencing the separation efficiency in the HPLC–direct injection method with an ODS column, such as mobile phase composition, column temperature and injection volume, were examined.

2. Experimental

2.1. HPLC system

The HPLC system was mainly composed of a pump (CCPM, Tosoh, or PU-980, Jasco), a sample injector (Rheodyne Model 7125 , sample size 200 μl–5 ml), a column oven (CO-965, Jasco) and a UV detector (UV-970, Jasco). A degassing unit and a solvent mixing unit were also installed. The columns were two Ultron VX-ODS (monomeric ODS, particle size 5 μm; Shinwa Chemical Industry), 150 mm × 4.6 mm I.D. or 250 mm × 4.6 mm I.D. A guard column (ODS, 5 μm, 10 mm × 4.6 mm I.D.) was fitted before the analytical column.

2.2. HPLC conditions

A mixture of acetonitrile (HPLC grade) and 1 m*M* phosphate buffer (pH 3–6) was used as the mobile phase, the phosphate buffer being prepared with ultra-pure water (18 MΩ/cm). The content of CH_3CN was varied in the range 30–60% in the isocratic elution mode and 30–80% in the gradient elution mode. The flow-rate was maintained at 1.5 ml/min.

The column temperature was set at 30°C. For the examination of the effect of temperature, the temperature was varied in the range 20–50°C. The most appropriate detection wavelengths of the UV detector suggested from the UV spectra of each pesticide were 220, 260 and 300 nm.

2.3. Pesticides

All pesticides listed in Table 1 were purchased as pesticide standards. Stock solutions of each pesticide were prepared at 500 mg/l with methanol (HPLC grade), except for iprodione, which was dissolved in acetonitrile because it decomposed in methanol [14]. The stock solutions were diluted with ultra-pure water for HPLC measurements: the high-level solutions were 0.03–1.0 mg/l, mid-level solutions were 3–100 μg/l and low-level solutions were 0.3–10 μg/l. 1,3-Dichloropropene was diluted just before HPLC measurements because of its high volatility. The stock solutions and aqueous standard solutions were stored in a refrigerator. The concentrations of the standard solutions for each pesticide are given in Table 1.

2.4. Effect of injection volume

For the direct injection method, the allowable injection volume is an important factor in determining the detection level. The effect of injection volume was examined with various sizes of sample loop (200 μl–5.0 ml).

2.5. Environmental samples

The environmental samples were obtained from a pond on a golf links in Aichi Prefecture, Japan, in April 1995. The water samples were injected directly into the HPLC system after filtration with a glass-fibre filter (rejected particle size 0.3 μm).

Table 1
List of pesticides used in this study

No.	Pesticide	Standard/ guideline (mg/l)	Standard solution (mg/l)			Group[a]	Detection level (μg/l)
			High-level	Mid-Level	Low-level		
1	Simazine (CAT)	0.003[b]	0.03	0.003	0.0003	A	0.05
2	Dichlorvos (DDVP)	0.01[c]	0.5	0.05	0.005	A	0.5
3	Thiram	0.006[b]	0.2	0.02	0.002	B	0.1
4	1,3-Dichloropropene	0.002[b]	1.0	–	–	B	–
5	Fenobucarb (BPMC)	0.2[c]	1.0	0.1	0.01	B	0.3
6	Propyzamide	0.008[c]	0.2	0.02	0.002	B	0.04
7	Iprofenfos (IBP)	0.008[c]	0.5	0.05	0.005	B	0.3
8	Isoprothiolane	0.04[c]	0.5	0.05	0.005	B	0.2
9	Chlorothalonil (TPN)	0.04[c]	0.2	0.02	0.002	B	0.3
10	Fenitrothion (MEP)	0.003[c]	0.3	0.03	0.003	B	0.4
11	Diazinon	0.005[c]	1.0	0.1	0.01	C	0.5
12	Isoxathion	0.008[c]	0.2	0.02	0.002	C	0.1
13	Thiobencarb	0.02[b]	0.3	0.03	0.003	C	0.2
14	EPN	0.006[c]	0.3	0.03	0.003	C	0.3
15	Chlornitrofen (CNP)	ND[d]	0.2	0.02	0.002	C	0.2
16	Asulam	–	0.5	–	–	A	–
17	Oxine-copper	–	0.5	–	–	A	–
18	Iprodione	–	0.2	–	–	B	–
19	Bensulide	–	0.5	0.05	0.005	C	0.2

[a] Mobile phase: (A) 30% CH_3CN; (B) 45% CH_3CN; (C) 60% CH_3CN.
[b] Standard.
[c] Guideline.
[d] Not detected.

3. Results and discussion

3.1. Separation conditions

The high-level solutions in Table 1 were used to investigate the separation conditions such as the mobile phase composition, pH, flow-rate, column temperature and wavelength using the 150-mm column.

The effect of the mobile phase composition was examined under the following conditions: CH_3CN 40–50% for thiram–chlorotalonil and iprodione (group B in Table 1) and CH_3CN 55–65% for diazinon–CNP and bensulide (group C in Table 1). The capacity factors of the pesticides decreased with increase in CH_3CN content, where the column void volume was measured by using $NaNO_3$ as a t_0 solute. From these relationships, the optimum CH_3CN content was concluded to be 45% for group B and 60% for group C. Although CAT and DDVP (group A in Table 1) were not well separated with the 45% CH_3CN mobile phase, 30% CH_3CN could separate them completely. Asulam and oxine-copper, however, were eluted faster than CAT and it was suggested that some other stationary phase such as a polymer gel [14] must be used for separating asulam and oxine-copper.

When the pH of the phosphate buffer was varied in the range 3–6, the retention times of all pesticides except oxine-copper were not influenced. Changes in flow-rate in the range 1–2 ml/min did not affect the separation factors. Based on these results, subsequent experiments were conducted using phosphate buffer of pH 6 at a flow-rate of 1.5 ml/min.

The effect of column temperature on the retention time is shown in Fig. 1. Higher temperatures gave shorter retention times but lower separation efficiency. For both groups B and C

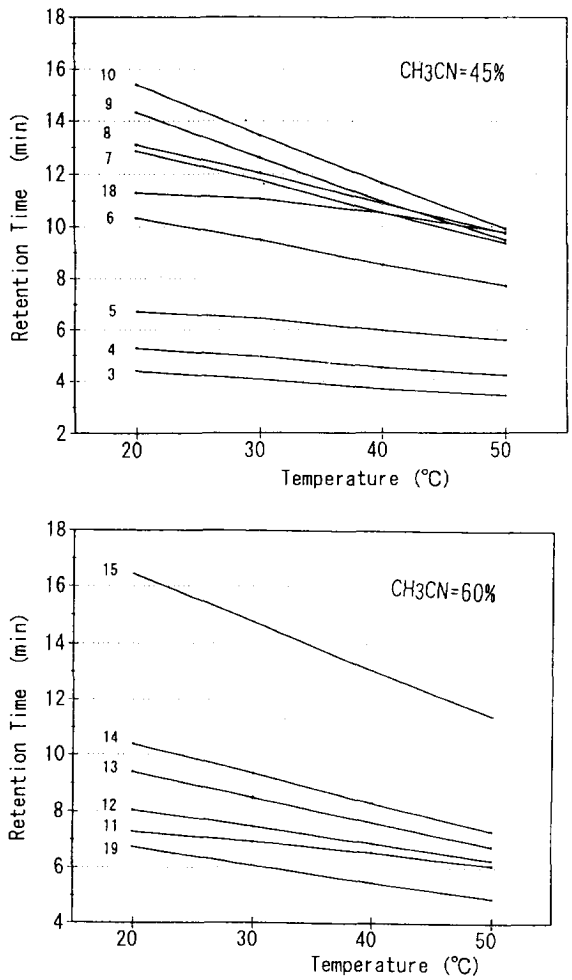

Fig. 1. Effect of the column temperature on retention. Column length, 150 mm; flow-rate, 1.5 ml/min; injection volume, 200 μl.

the optimum column temperature was between 25 and 30°C.

The chromatograms obtained under the above isocratic conditions are shown in Fig. 2. *Cis*- and *trans*-1,3-dichloropropene were not separated under these conditions. However, it is not necessary to separate these isomers, because the total concentration of isomers is the regulatory requirement. Isoprothiolane and iprodione were not also separated. Isoprothiolane included in the guidelines was found to be detected preferentially at 300 nm; the peak intensity of iprodione at 300 nm was less than 1% in comparison with that for isoprothiolane at the same concentration. Thiobencarb was also detected with much higher sensitivity at 260 nm. The chromatograms with UV detection at 300 and 260 nm are shown in Fig. 3.

3.2. Simultaneous separation

Since more rapid analysis requires simultaneous separation for these pesticides, several gradient elution conditions were examined using the high-level solutions. When a 150-mm column was used, IBP–isoprothiolane and bensulide–diazinon were not completely separated under linear gradient elution conditions. When a 250-mm column was used, however, all pesticides except iprodion were completely separated under gradient conditions with the CH_3CN content increased linearly from 30% to 80% in 60 min at a flow-rate of 1.5 ml/min.

Fig. 4 shows the chromatogram for an injection volume of 200 μl. In this case *cis*- and *trans*-1,3-dichloropropene were slightly separated. Complete separation was also obtained with a 500-μl injection. With a 1.0-ml injection, the peak width increased by a factor of more than 2 and, therefore, bensulide–diazinon and IBP–isoprothiolane were not separated. This may be caused by the fact that the 1-ml loading on the column requires 40 s at a flow-rate of 1.5 ml/min. A large-volume injection may affect the mobile phase composition in the column with gradient elution conditions and therefore some modification of the gradient conditions may be required for a large-column injection.

3.3. Effect of injection volume

When 500 μl of the low-level standard solutions were injected, all pesticides were detected. In HPLC, however, it is necessary to introduce a large volume of sample to improve the detection level because of the sensitivity limitation of the UV detector.

The effect of the injection volume on the separation performance was examined under isocratic elution conditions. The injection volumes were varied using the injection loop. Even

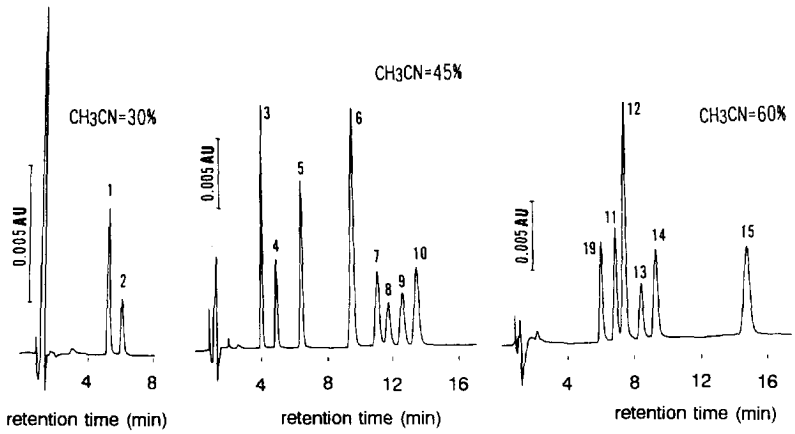

Fig. 2. Chromatograms for high-level solutions with UV detection at 220 nm. Column length, 150 mm; temperature, 30°C; flow-rate, 1.5 ml/min; isocratic mode.

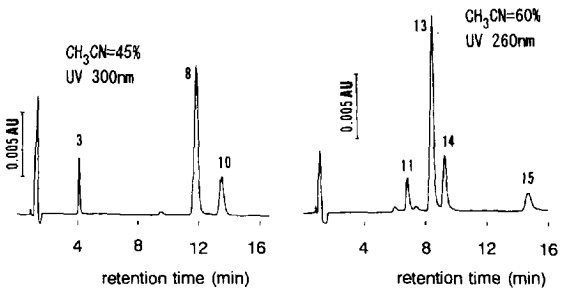

Fig. 3. Chromatograms for high-level solutions with UV detection at 260 or 300 nm. Column length, 150 mm; temperature, 30°C; flow-rate, 1.5 ml/min; isocratic mode.

when a 5-ml sample was injected, good chromatograms were obtained. The theoretical plate numbers were $21 \cdot 10^3$ for group A, $7 \cdot 10^3$–$10 \cdot 10^3$ for group B and $11 \cdot 10^3$–$13 \cdot 10^3$ for group C.

In this case the theoretical plate number was varied significantly with the injection volume, because a 5-ml loading on the column requires 200 s at a flow-rate of 1.5 ml/min and the retention times increased with increase in loading time. Therefore, the separation efficiency was evaluated by the resolution of the critical pair. The critical pairs used here were the same for all

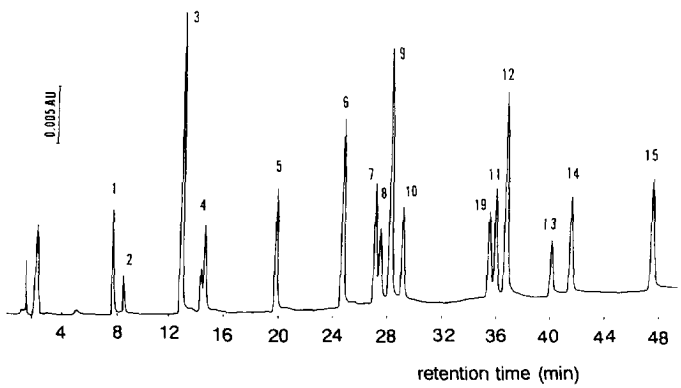

Fig. 4. Simultaneous separation of pesticides with UV detection at 220 nm. Column length, 250 mm; temperature, 30°C; elution, gradient mode from 30% to 80% CH_3CN in 60 min; flow-rate, 1.5 ml/min; injection, high-level solution, 200 μl.

Table 2
Resolutions for the critical pairs

Critical pair[a]	Injection volume				
	200 μl	500 μl	1.0 ml	2.0 ml	5.0 ml
7 and 8	1.60	1.47	1.21	1.21	1.06
11 and 12	1.77	1.72	1.67	1.57	1.57

[a] For identification, see Table 1.

different injection volumes such as IBP and isoprothiolane in group B ([CH_3CN] = 45%) and diazinon and isoxathion in group C ([CH_3CN] = 60%). The resolutions (R_s) for the critical pairs were calculated and are given in Table 2. Although the resolution for group C remained at relatively high level, that for group B decreased more.

These results confirmed that the direct injection method may permit a large injection volume of up to 5 ml. When the pesticides in a water matrix were introduced into the column, the pesticides were first adsorbed strongly on the guard column and/or the front part of the analytical column followed by separation with the mobile phase. In comparison with the common solid-phase extraction method, this method has the advantage that all amounts of pesticides in the samples can be introduced into the column.

3.4. Environmental samples

The water obtained from a pond on a golf links was measured as an example of the analysis of environmental samples using the isocratic elution mode. The water sample was filtered with a glass-fibre filter (0.3 μm) and 5.0 ml were injected. The chromatograms for the environmental sample and the low-level standard solutions are shown in Fig. 5. The noise level for the environmental sample was estimated to be ca.

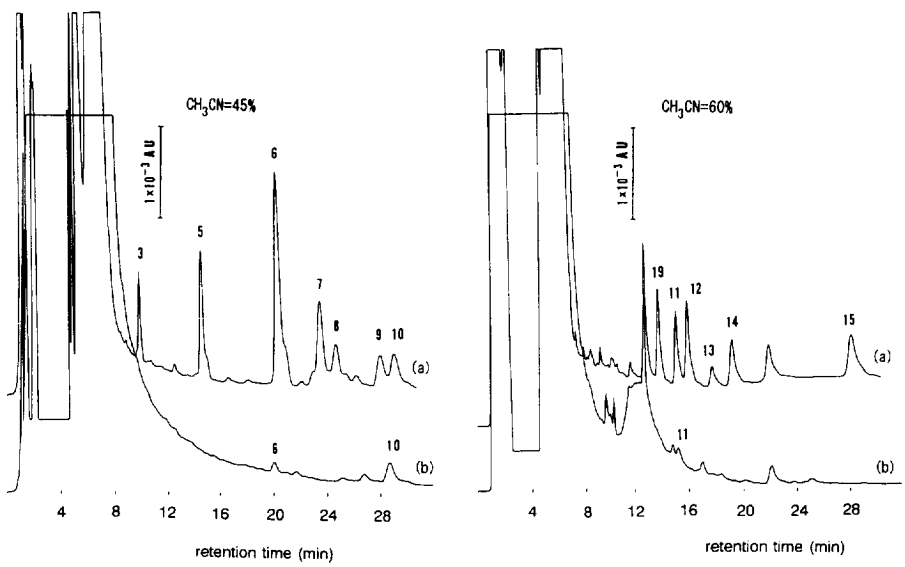

Fig. 5. Chromatograms for the environmental water sample and the low-level standard solutions. Column length, 250 mm; temperature, 30°C; flow-rate, 1.5 ml/min; isocratic mode; injection volume, 5.0 ml.

$7 \cdot 10^{-6}$ absorbance from measurements under high-sensitivity conditions of the UV detector. When the detection limit was assumed to correspond to $S/N = 3$, the peak height corresponds to 0.5% of full-scale in Fig. 5. Although the sensitivity of the UV detector can be increased more, the detection limit was assumed to be 1% of full-scale for Fig. 5 and the detection limit for each pesticide was evaluated as shown in Table 1. The lowest detection limit was 0.05 μg/l for simazine and the highest 0.5 μg/l for DDVP and diazinon.

In the case of the 30% CH_3CN mobile phase, no significant peak was seen for the environmental sample. When the concentration of CH_3CN in the mobile phase was 45% or 60%, popyzamide, MEP (group B) and diazinon (group C) were detected, and their concentrations were estimated to be ca. 0.07, 1.3 and 1.1 μg/l, respectively. Some other unidentified peaks were also observed in the environmental sample. Since the information on retention times is not sufficient for the identification of the pesticides in environmental samples, a diode-array UV detector must be recommended for practical environmental analysis.

Pesticide analysis is also an important requirement in water works. Engineers in water works must monitor the water quality and confirm that it is up to the required standard. If the pesticide concentrations evaluated by this method are lower than the standard, the water may be accepted as potable water. On the other hand, if some peaks are observed that are higher than the standard levels, the identification of these peaks is required in order to clarify the pollution source of the pesticides. In this respect, the direct injection method may be of use as a rapid screening procedure. In addition, this method may be also suitable for the examination of pesticides removal processes by physico-chemical treatment such as activated carbon adsorption and membrane filtration.

4. Conclusion

The described direct injection method in HPLC is of use for the determination of pesticides in water and showed the following attributes: (1) in the gradient elution mode, sixteen pesticides were well separated, although the allowable injection volume was less than 500 μl; (2) in the isocratic elution mode, good chromatograms were obtained even with a 5-ml injection and the pesticides were detected at the sub-ppb level; and (3) environmental samples were analysed directly only after a filtration step.

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