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On-line coupling of solid-phase extraction to gas chromatography with fast solvent vaporization and concentration in an open injector liner
Analysis of pesticides in aqueous samples

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Abstract

The purpose of this study is to combine solid-phase extraction (SPE) with gas chromatography (GC) for the fully automated determination of pesticides and herbicides in aqueous samples. The interface technique employed for connecting SPE with GC was fast solvent vaporization and concentration in an open injector liner. The interface device consisted of the programmed-temperature vaporizing injector without using the packing material in the liner and the target compounds were concentrated around the inlet of the GC capillary column. This avoided the degradation of target compounds, and no precise control of the injecting speed was required, when an automatic SPE system was connected to GC–MS. The aqueous samples used in this system were prepared by spiking 29 kinds of pesticide and herbicide compounds, which are regulated by the Ministry of Health and Welfare of the Japanese National government, in purified water and river water, to a resulting concentration of 1 μg/l. Employing this system, the recoveries and RSDs ($n=6$) of most compounds were greater than 75% and within 10%, respectively. From the results of this study, we found that on-line automatic SPE and capillary GC–MS equipped with the fast solvent vaporizing and concentrating method in an open injector liner could be connected in order to obtain good results for the determination of pesticides in water samples.

Keywords: Interfaces, SPE–GC; Water analysis; Environmental analysis; Automation; Pesticides

1. Introduction

In the conventional methods for the determination of pesticides and herbicides in tap water and river or lake waters, the eluate obtained in the sample preparation methods such as solid-phase extraction (SPE) and liquid–liquid extraction is injected onto a gas chromatography–mass spectrometry (GC–MS) system manually. However, such methods are likely to cause human errors and require operator's technical skills. In such circumstances, the development of a fully automatic method has been tried by coupling of the automatic sample preparation device with an analytical instrument [1–3].

With conventional capillary GC, an injection volume of only 2 μl was possible, which was unacceptable as a sample volume as large as 10–100 μl eluted by an automatic SPE device was injected. In recent years, large-volume injection methods have been developed, and increasing research on the coupling of SPE to GC has been reported. PTV (programmed-temperature vaporizing) large-volume

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injection [4] and on-column large-volume injection [5–7] have been applied as the typical interface connecting SPE to GC. These interface techniques, however, have some disadvantages as follows. In the PTV method using a packed liner tube, some target compounds are decomposed due to the catalytic effects of the packing material. Some kinds of the target compounds are too strongly retained on the packing particles in the liner [8,9], therefore, they cannot be desorbed from the packing particles. In the latter method, a long pre-column and a solvent vent line are used, and injecting speed must be precisely controlled [10,11]. In order to solve the problems, we developed a new interface technique. The interface device consisted of a PTV injector without using the packing material in the liner and the target compounds were concentrated around the inlet of the GC capillary column. This avoided the degradation of target compounds, and no precise control of the injecting speed was required, when an automatic SPE system was connected to GC–MS. This system was applied to determine 29 kinds of pesticide and herbicide compounds in aqueous samples.

2. Experimental

2.1. Chemicals and reagents

All standard compounds of pesticides and herbicides were from GL Sciences (Tokyo, Japan). They are regulated by the Ministry of Health and Welfare of the Japanese National government. Acetone and methanol of pesticide residue analysis grade were purchased from Kishida (Tokyo, Japan). A standard solution of 1000 mg/l of each compound was prepared in acetone and stored in the refrigerator. A mixture solution of 0.1 mg/l in acetone was prepared from the standard solutions and spiked into water samples at the required concentrations. Reagent water was obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA). River water samples were filtered through a 1.0-μm glass fiber filter GA-100 (Advantec Toyo, Tokyo, Japan).

Helium and nitrogen were supplied by Nissan Syoji (Tokyo, Japan) with a purity of 99.995%.

2.2. SPE instrument system

The automatic SPE instrument system consisted of a Prospekt system (Spark Holland) for sample preparation, a MIDAS autosampler (Spark Holland) and a PU610 HPLC pump (GL Sciences) for eluting the target compounds from the SPE cartridge.

The Prospekt system consisted of three pneumatic six-port valves, the automatic cartridge exchanger and the solvent delivery unit (SDU) equipped with the six-port solvent selection valve and a single-piston HPLC pump. All timed events were programmed via a software package into the Prospekt controller unit. A stainless steel sample loop of 2 ml was attached to the sampling valve of the MIDAS autosampler.

The SPE cartridge employed in the Prospekt system was of 10 m×2.0 mm I.D., packed with 15–25 μm particle diameter styrene–divinylbenzene copolymer PLRP-S (Spark Holland). The outlet of the SPE valve and the GC injector are connected using a deactivated capillary tube of 0.7 m×0.25 mm I.D. The nitrogen gas was purified with an active carbon trap prior to being provided to the system. The schematic flow diagram of this automated SPE system is shown in Fig. 1.

2.3. GC instrument

The GC instrument used was a HP6890 gas chromatograph (Hewlett-Packard) equipped with an OPTIC 2-200 PTV injector (ATAS, The Netherlands) and a HP 5973 mass-selective detector (Hewlett-Packard). The liner (was 80 mm×5 mm O.D.) used in the PTV injector was an open injector liner for fast solvent vaporizing and concentrating (GL Sciences). The pre-column used was the deactivated capillary tube; 0.3 m×0.53 mm I.D. (GL Sciences). The analytical capillary column was an NB-5 (5% phenyl–methylsiloxane) of 30 m×0.25 mm I.D., film thickness; 0.4 μm (GL Sciences). The pre-column and the analytical capillary column are connected using the press-fit connector.

2.4. SPE conditions

At first the sample loop was cleaned and con-

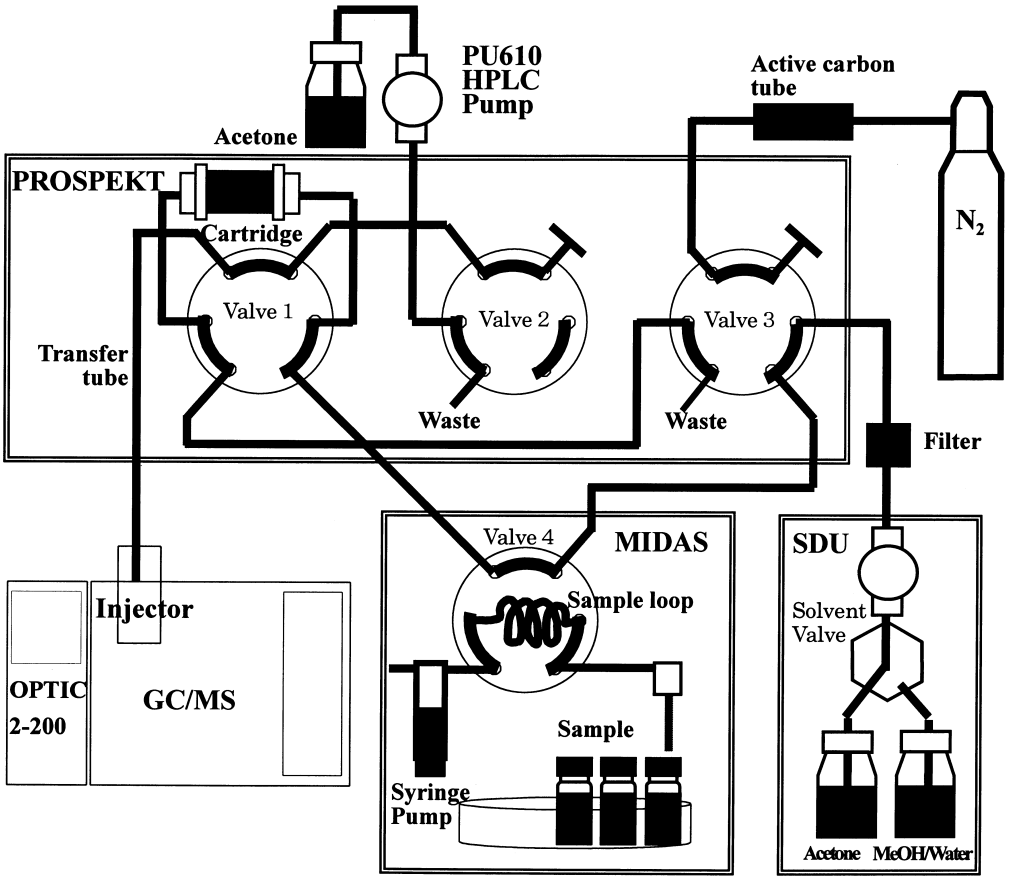

Fig. 1. Schematic flow diagram of automated on-line SPE–GC–MS system.

ditioned with 2 ml of acetone and 4 ml of 30% MeOH in water. Then, during sampling of a 2-ml water sample with MIDAS, the SPE cartridge was cleaned and conditioned with 2 ml of acetone and 3 ml of 30% MeOH in water. The 2-ml sample was loaded onto the SPE cartridge at a flow-rate of 1 ml/min, and it was cleaned up to remove ionic and very polar compounds with 30% MeOH in water. In the next step, the cartridge was dried for 24 min with 50 ml/min nitrogen gas at ambient temperature to remove the water from the cartridge. The analytes trapped in the cartridge were eluted with 20 μl of acetone that was pumped at the rate of 100 μl/min using the PU610 HPLC pump and transferred to the GC system through the transfer line. The time program of the SPE system is shown in Table 1.

2.5. Transferring procedures

In this study, the eluate from SPE was sandwiched between pre-solvent (i.e., the transfer of organic solvent into the GC system prior to the eluate) [12] and post-solvents (i.e., after the eluate) and then was transferred to the GC system. Solvent remaining in the transfer tube was pushed out by nitrogen gas, and was completely removed by reversed-flashing of the carrier gas (He) as follows. After removing water in the SPE cartridge by the nitrogen gas purge, 5 μl of pre-solvent was introduced through the transfer tube so that target compounds would not be adsorbed on the dry inside walls of transfer tube and other flow lines. After an eluate, 5 μl of post-solvent was introduced so that target compounds would not

Table 1
Time program of SPE system

Time (min:s)	SDU		MIDAS,	Prospekt[b]							Event
	Solvent valve[a]	Flow (ml/min)	Value 4	Valve 1	Valve 2	Valve 3	Change cartridge	Auxiliaries[c]			
								1	2	3	
00:00	1	2.0		2	2	2					Cleaning loop with acetone
01:00	2										Cleaning loop with water
02:30									On		MIDAS start
02:32									Off		
03:00	1										Conditioning cartridge with acetone
04:00	2										Conditioning cartridge with MeOH–water
05:30		1.0									Change flow
06:00										On	Concentrating
11:00	0	0.0				1				Off	Drying cartridge with N_2 gas
35:00				1	1	2					Pre-elution
35:50				2							Pre-solvent
35:53				1							Elution
36:18				2		1					Post-solvent
36:21				1		2					Removing the solvent in the transfer tube
36:50	1	1.0		2	2						Concentrating an eluate in open liner
38:59				1							
39:00							Change				Change a cartridge and back flash
39:01				2				On			Optic and GC–MS start
39:03								Off			
41:00	2										Cleaning
45:00	0	0.0									Stop
48:00											End time

[a] 1, Acetone; 2, MeOH–water.
[b] V1–V4: position 2 refers to position in Fig. 1.
[c] 1, Optic start o/p; 2, MIDAS freeze o/p; 3 MIDAS injection.

remain in the transfer tube (Fig. 2a). While the post-solvent was introduced, the cartridge was filled with nitrogen gas by purging. Then, by pouring a solvent into the cartridge again, the eluate and solvents in the transfer tube were injected into the liner of the GC system with the extruding nitrogen gas. The line was closed to prevent backflow until the target compounds were concentrated in the liner (Fig. 2c). When they were concentrated, the SPE cartridge was exchanged so that the solvent remaining in the transfer tube could be completely removed by reversed-flashing of the carrier gas (He) (Fig. 2d).

2.6. Chromatographic conditions

The initial injector temperature, oven temperature and GC carrier gas pressure were set at 60°C, 75°C and 25 kPa, respectively. The initial conditions were maintained until the eluate had been transferred and concentrated in the liner. Then, the injector temperature was increased to 280°C at 2°C/s. The oven temperature was increased to 200°C at 10°C/min after 2 min, to 250°C at 5°C/min, to 290°C at 10°C/min and then kept at 290°C for 6.5 min. The GC carrier gas (helium) pressure was increased to 50 kPa, after 2 min, to 200 kPa at 4.5 kPa/min. Split and vent purge flow-rate was set at 30 ml/min during the operation. The MS transfer line was kept at 280°C. The electron impact (EI) ionization conditions were ion energy 70 eV. Selected ion monitoring (SIM) acquisition was carried out by acquiring data from the ions in Table 3.

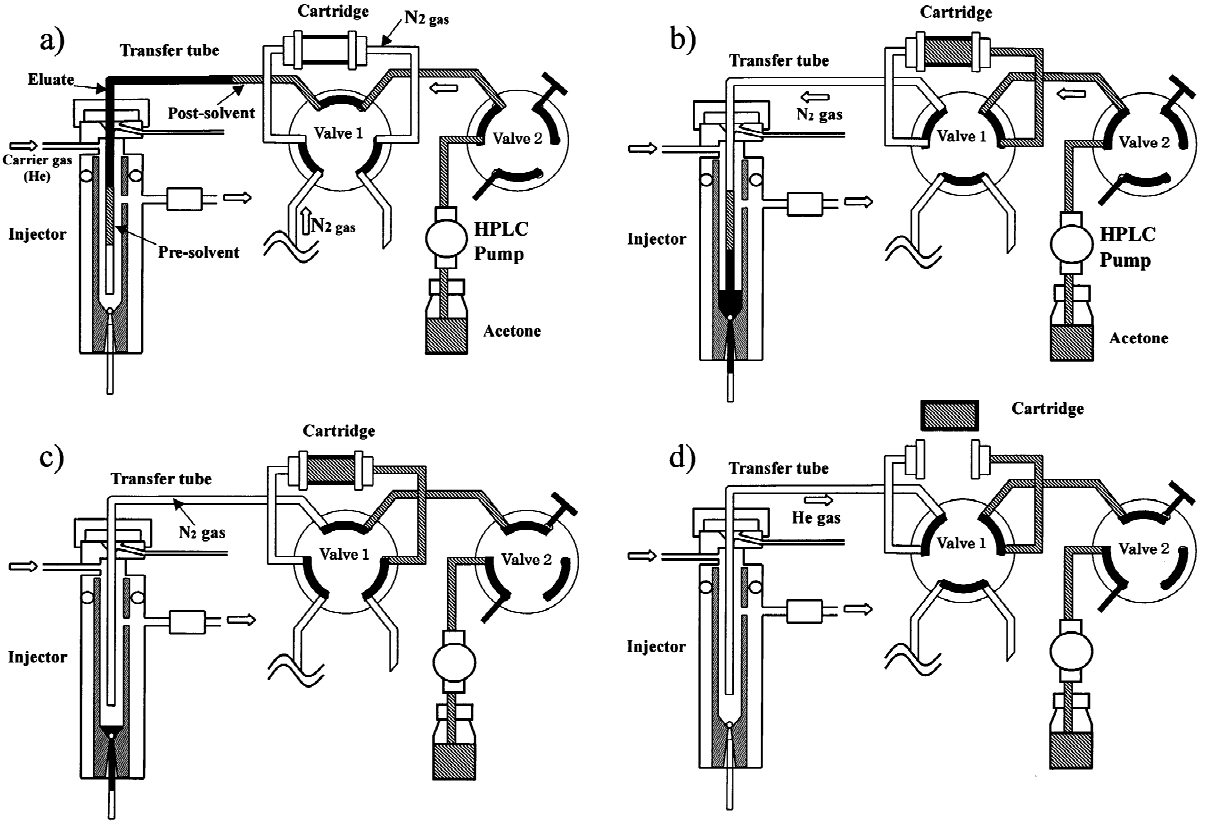

Fig. 2. Schematic flow diagram of transfer procedures. (a) Sandwiching an eluate between pre-solvent and post-solvent; (b) injecting the eluate and solvents into the liner; (c) concentrating the target compounds in the liner; (d) removing the remaining solvent completely by reverse flashing when the cartridge exchanged.

3. Results and discussion

3.1. Fast solvent vaporizing and concentrating in the open injector liner

The injector was kept at a temperature lower than the boiling point of the sample solvent and the column oven was set at a temperature higher than the boiling point of the sample solvent. The sample solution was injected into the liner as shown in Fig. 3. The solvent stayed in the liner, if the carrier gas pressure and the solvent vapor presser could be equilibrated. While the evaporated solvent was exhausted, the target compounds were concentrated around the inlet of the GC capillary column. Then, the target compounds were separated on the analytical column at an elevated column oven temperature. This large-volume injection method was employed for an interface to connect an SPE system to GC–MS as an automatic on-line operation system. In this system, the eluate from a SPE cartridge was automatically injected into the GC–MS capillary system. The outlet of the liner was connected to the pre-column, which was a deactivated silica capillary tube, using a press-fit connector. The liner had a small hole, which was used as a vent for the evaporated sample solvent and was located at a sufficiently high level for filling of a large volume of the introduced sample. In order to prevent that a plug of sample solvent was vibrated between the liner and

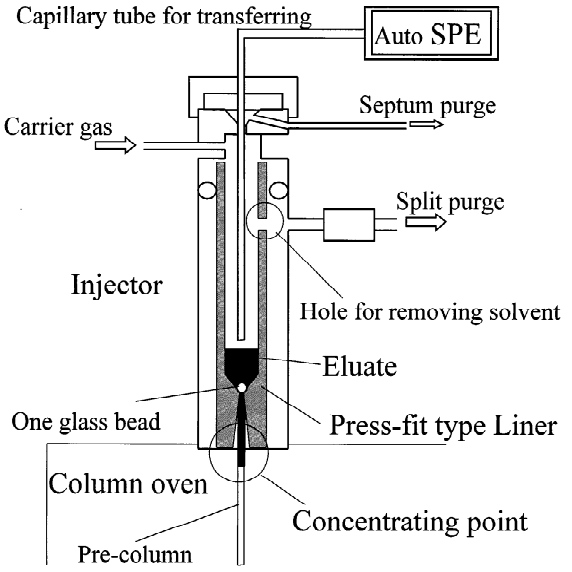

Fig. 3. Scheme of on-line interface employed for fast solvent vaporizing and concentrating in the open injector liner.

the capillary column, a glass bead (1 mm O.D.) was placed at the bottom of the liner, which led to an increase in the flow resistance at the outlet of the liner.

This method avoided the degradation of target compounds, and no precise control of the injecting speed was required, when an automatic SPE system was connected to GC–MS. In this study, during transfer and evaporation of the sample solvent, the temperature of the injector was set at 60°C and the column oven temperature was set at 75°C, since the boiling point of acetone is 63°C (at 25 kPa).

3.2. Optimum concentration of adding MeOH to the loading water

Though purified water was used for loading and clean-up, some compounds obtained low recoveries. It was guessed that the target compounds might be adsorbed in the flow lines and valves [13]. The sample flow lines used were stainless steel tubes. The valves were made of SUS 316, poly(vinylidene difluoride) (PVDF) and Vespel. So, collecting the adsorbed target compounds was tested by adding MeOH to the water. Recoveries obtained by adding different concentrations of MeOH and log K_{OW} (octanol–water partition coefficient) values [14] of each compound are shown in Table 2. For benfluraline, chlorpyrifos, pendimethalin, butamifos, chlornitrofen, pyributicarb and ethyl p-nitrophenyl phenylphosphonothioate (EPN), a decrease in recoveries appeared in the range of low concentration of MeOH. This suggests that these compounds were adsorbed on the valve and flow lines when water sample loading because their log K_{OW} values are high (beyond 4.5). Therefore, the adsorbed target compounds could be collected by adding MeOH to the purified water for loading. Higher recoveries could be obtained for most compounds by using 30% and more of MeOH. For dichlorvos and simazine, a decrease in recovery appeared in the range of high concentration of MeOH. It was considered that these compounds could not be retained in the packed material during water sample loading and clean-up due to low log K_{OW} values (less than 2.5). Therefore, we selected 30% MeOH as an optimum concentration.

3.3. The required volume of acetone for eluting from SPE cartridge

We examined the recoveries of pesticides concentrated on the PLRP-S cartridge, changing the volume over a range of 10, 20, 30 and 60 μl acetone for eluting target compounds from the SPE cartridge. It was found that most pesticides were completely eluted when using a volume of acetone larger than 10 μl. On the other hand, [$^2H_{10}$]phenanthrene was more strongly absorbed and required a larger amount of acetone (>20 μl). Therefore, we selected 20 μl as the required volume of acetone in this method.

3.4. Recovery and reproducibility

The performance of this system was evaluated by determination of pesticides in purified water and river water. First, a blank of these samples was analyzed to check whether there were any peaks in the corresponding chromatogram at retention times similar to those of the pesticides. The samples used were prepared by spiking the target compounds to these waters at a concentration of 1 μg/l. The results are shown in the Table 3. The recoveries and RSDs (n=6) were calculated from the obtained chromato-

Table 2
Recoveries (%) obtained by adding different concentrations of MeOH to the loading purified water[a]

No.	Compound	Recovery (%)					Log K_{OW}
		MeOH (%)					
		0	10	20	30	40	
1	**Dichiorvos**	**134.9**	**128.8**	**136.0**	**116.3**	**6.4**	**1.9**
2	Etridiazole	151.4	162.8	164.2	172.1	145.5	3.4
3	Chloroneb	103.5	110.8	106.3	112.9	104.0	–
4	Fenobucarb	131.4	127.4	136.0	134.7	131.7	2.8
5	***Benfluraline***	***35.5***	***58.6***	***65.6***	***71.6***	***85.8***	***5.3***
6	***Pencycuron***	***78.9***	***99.6***	***91.2***	***107.5***	***102.3***	***4.7***
7	**Simazine**	**109.6**	**104.8**	**109.0**	**103.1**	**42.0**	**2.1**
8	Benzene hexachloride (BHC)	97.9	105.2	99.5	105.7	98.8	3.7
9	Propyzamide	104.2	103.3	107.5	109.7	109.0	–
10	[$^2H_{10}$]Phenanthrene	61.8	71.4	66.3	77.5	77.0	–
11	Diazinon	77.4	90.6	81.5	85.3	87.0	3.3
12	Chiorothalonil	101.3	99.0	96.1	94.2	94.0	2.9
13	Iprobenfos	106.1	120.5	119.4	124.1	126.5	3.2
14	Terbucarb	101.7	105.0	103.1	104.1	104.8	–
15	***Tolciofos-methyl***	***76.4***	***93.5***	***85.2***	***94.8***	***93.9***	***4.6***
16	**Metalaxyl**	99.3	**95.1**	**100.1**	**100.5**	**93.1**	**1.8**
17	Fenitrothion	95.7	113.0	121.9	128.9	134.5	3.5
18	***Chlorpyrjfos***	***53.3***	***78.2***	***74.1***	***84.7***	***88.0***	***4.7***
19	***Pendimethalin***	***42.3***	***74.5***	***73.4***	***92.2***	***99.1***	***5.2***
20	Methyldymron	123.2	106.8	119.5	121.5	118.1	3.0
21	Isofenphos	55.2	69.0	53.4	47.5	48.4	4.0
22	***Butamifos***	***52.0***	***75.2***	***65.0***	***63.9***	***62.0***	***4.6***
23	Flutolanil	83.1	85.8	91.3	91.7	96.0	3.7
24	Napropamide	88.2	98.6	93.4	98.6	96.2	3.3
25	Isoprothiolane	70.2	82.7	70.5	76.3	82.3	3.3
26	Mepronil	98.1	107.8	111.1	117.9	123.1	3.7
27	***Chiornitrofen***	***39.3***	***63.9***	***66.4***	***86.3***	***90.5***	***5.1***
28	***Pyributicarb***	***26.9***	***51.9***	***41.9***	***41.5***	***33.1***	***5.2***
29	Pyridaphenthion	73.3	76.4	89.8	98.0	106.6	3.2
30	***EPN***	***45.8***	***67.0***	***66.5***	***80.8***	***85.1***	***4.6***

[a] Bold, log K_{OW}<2.5; italic bold, log K_{OW}>4.5; –, unknown. The recovery was calculated by (the peak areas obtained using this on-line method/the peak areas obtained using the direct injection). Direct injection; we injected 20 μl sample solution at a concentration of 0.1 μg/μl to the GC system directly.

grams. The recoveries of most compounds were greater than 75%. RSDs were approximately within 10%. However, obtained recoveries of etridiazol, fenitrothion and pyridaphenthion were extraordinarily high (>130%) in river water. This was considered the effect the river water matrix supposedly this led to higher recoveries than purified water. Chlorothalonil was obtained in low recoveries and at low reproducibility in river water. We considered that it was decomposed by contamination in the liner. The SIM chromatogram of 1 μg/l pesticides and herbicides spiked to river water is shown in Fig. 4. Peak shapes were satisfactory, except for dichlorvos, etridiazol and chloroneb. These shapes became poorer as this system ran, which indicated that the problem is caused by contamination in the liner and pre-column.

4. Conclusion

An automatic SPE system was connected to GC–MS, using fast solvent vaporizing and concentrating in an open injector liner. This system was applied to the fully automated determination of pesticides and herbicides in the aqueous samples.

Table 3
Recoveries and RSDs (%, $n=6$) obtained using this on-line system[a]

No.	Compound	Monitor ion (m/z)		t_R (min)	Purified water		River water	
		Target	Qualifier		RSD (%)	Recovery (%)	RSD (%)	Recovery (%)
1	Dichiorvos	185	109	9.59	5.67	101.6	7.75	88.2
2	Etridiazole	211	183	12.55	4.96	128.0	4.57	160.6
3	Chioroneb	191	206	13.21	2.62	110.3	1.80	112.4
4	Fenobucarb	121	150	14.41	3.04	111.9	4.57	127.1
5	Benfluraline	292	264	15.23	6.98	67.3	10.45	91.5
6	Pencycuron	180	209	15.36	3.82	90.6	5.35	112.1
7	Simazine	201	186	15.97	4.12	98.6	4.65	106.1
8	Benzeme hexachloride (BHC)	219	181	16.46	1.97	107.8	3.02	108.4
9	Propyzamide	173	255	16.57	3.40	98.4	5.60	111.6
10	[$^2H_{10}$]Phenanthrene	188	187	16.69	3.16	90.9	3.59	82.7
11	Diazinon	304	179	16.75	2.95	97.5	4.76	95.5
12	Chiorothalonil	266	264	17.16	6.63	81.0	27.59	46.7
13	Iprobenfos	204	288	17.34	2.15	103.5	5.96	125.3
14	Terbucarb	205	220	17.98	3.46	102.2	5.73	112.6
15	Tolclofos-methyl	265	267	18.19	3.47	106.5	4.13	107.2
16	Metalaxyl	206	220	18.36	2.53	97.2	4.81	104.0
17	Fenitrothion	277	260	18.78	2.60	95.2	10.00	138.8
18	Chiorpyrifos	314	197	19.38	6.18	92.5	4.87	103.7
19	Pendimethalin	252	281	20.31	9.20	77.7	7.67	114.7
20	Methyldymron	107	119	20.48	7.81	127.1	5.77	124.8
21	Isofenphos	213	255	20.55	5.76	80.3	7.05	70.5
22	Butamifos	286	200	21.73	6.39	77.0	11.88	94.4
23	Flutolanil	173	323	21.76	4.67	87.0	5.05	100.4
24	Napropamide	271	171	21.77	2.54	96.9	3.93	102.4
25	Isoprothiolane	290	189	21.90	2.47	91.6	4.50	88.8
26	Mepronil	119	269	23.75	2.84	82.1	5.94	124.5
27	Chiornitrofen	317	236	24.16	10.56	77.5	7.75	108.6
28	Pyributicarb	165	181	25.66	13.55	59.8	9.12	74.3
29	Pyridaphenthion	340	199	25.86	3.11	96.9	7.86	141.7
30	EPN	157	323	26.07	8.83	81.5	10.18	105.9

[a] The sample was 1 μg/l pesticides and herbicides spiked to the purified water and river water. The recovery was calculated by (the peak areas obtained using this on-line method/the peak areas obtained using the direct injection method). Direct injection; we injected 20 μl sample solution at a concentration of 0.1 μg/μl into the GC system directly.

The target compounds adsorbed on the valve and flow lines of the SPE system could be collected by 30% MeOH in water for loading sample. Most compounds were completely eluted with 20 μl acetone from the SPE cartridge. The eluate was sandwiched with solvents and then was introduced into the GC system. The interface device employed for connecting SPE with GC consisted of the PTV injector without using the packing material in the liner and the target compounds were concentrated around the inlet of the GC capillary column. Finally, the compounds were separated on the analytical column by elevating column oven temperature such as cool on-column injection. This interface technique avoided the degradation of target compounds, and no precise control of the injecting speed was required, when an automatic SPE system was connected to GC–MS. The recoveries and RSDs of most of the compounds were satisfactory.

As the result of this study, we found that by on-line coupling of SPE to GC by the interface technique of fast solvent vaporizing and concen-

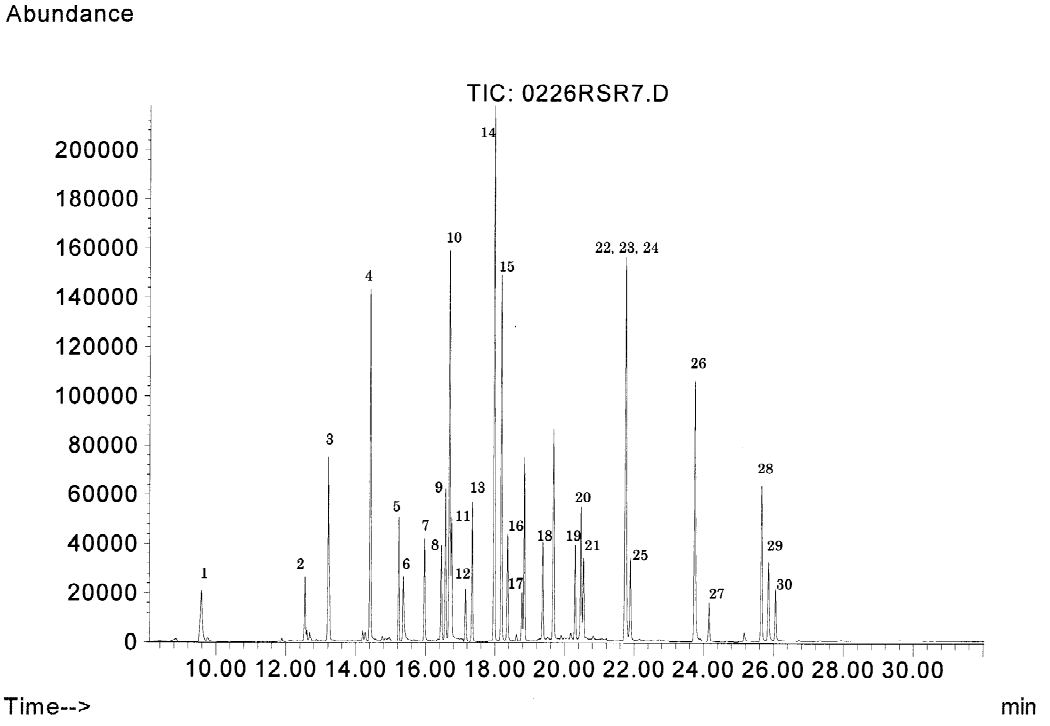

Fig. 4. GC–MS chromatogram of 1 μg/l pesticides and herbicides spiked to river water obtained employing this on-line system. For peak assignment, see Table 3. Time scale in min.

trating in an open injector liner, good results could be obtained for the determination of pesticides in water samples.

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