Simple and Rapid Determination of Golf Course Pesticides by In-Tube Solid-Phase Microextraction Coupled with Liquid Chromatography

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A simple and rapid method for the simultaneous determination of seven golf course pesticides in aqueous samples was developed by using in-tube solid-phase microextraction (in-tube SPME) coupled with liquid chromatography. In-tube SPME, in which the analytes were extracted from the sample directly into an open capillary column, is an extraction technique for organic compounds in aqueous samples. Although an open tubular capillary column with a thick film of polymer was used for the conventional in-tube SPME, we used a porous-layer open-tabular (PLOT) column in which there was a porous layer on the inner wall and has a larger surface area. A microsyringe pump equipped with a gastight syringe was employed to sequentially pump the sample solution through the capillary. The detection limits were from 0.9 to 4.1 ng ml^{-1}. The calibration curves were linear in the range from 1 to 50 ng ml^{-1}. We took a survey of seven golf course pesticides in several water samples by using the developed method.

Key words ——— liquid chromatography, capillary column, pesticide, organic compound

INTRODUCTION

The number of industrially produced chemical substances is estimated to be in the tens of thousands. Chemical substances have become indispensable in our daily lives, but on the other hand, they may affect human health and the ecosystem. The pollution of food and environment caused by them is one of the most important problems for human beings. Civic concerns have become very high at substances, such as dioxins and endocrine disruptors, which affect human beings in a very small quantity. And the concern about the residual pesticides in food and the agricultural chemicals which flow out of farmland or golf courses is also high. In the 1980s, the report to which the caddie and the golfer complained of healthy damage, such as a rash and pain of an eye, or the fish died in large quantities on the river near the golf course was carried out. Moreover, it has also been worried that the agricultural chemicals which flowed out may pollute a surrounding river and groundwater. The Ministry of the Environment (MOE) has established in 1990, "The Guidelines for Prevention of Water Pollution by Agricultural Chemicals used on golf courses to promote countermeasures" and the situation is now being considerably improved. The amount of golf course pesticides used in 2002 fiscal year in Chiba Prefecture was 112.7 t, and was decreasing from 316.2 t of 1989 fiscal year. In recent years, according to our survey by the regular method, few kinds were detected and their concentrations were almost 1 ug l^{-1}. However, Mecoprop was detected at 28 ug l^{-1} from a golf course runoff in the 2000 fiscal year. This concentration is equal to 56% of the guideline value, and may affect the ecosystem. And mecoprop is a chlorophenoxy-herbicide which, as a group, has been classified as possibly carcinogenic to humans. Additionally the number of golf course pesticide has increased from 35 to 45, we should study about pesticides added newly. We think that it is necessary to investigate those in runoff and water environment so that human health or ecosystem may not be affected further.

The sample which must be analyzed is increasing and very rapid, but still selective and sensitive systems are required. So we tried to develop a simple

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and rapid measurement method for golf course pesticides. An ideal extraction method should be rapid, simple, inexpensive, and give reproducible and high recoveries. Conventional solvent extraction techniques need a large amount of organic solvent and are time-consuming and labor-intensive. Solid-phase extraction (SPE) needs less solvent but it is a time-consuming multi-step process. A new approach to sample preparation, solid-phase microextraction (SPME), was invented by Pawliszyn and co-workers in 1989 for attempting to redress limitations inherent in SPE and LLE.[1] And it has been routinely used in combination with gas chromatography (GC) and GC/mass spectrometry (GC/MS).[2–6] But analyzing weakly volatile or thermally labile compounds are not amenable to GC or GC/MS.

Recently a new system known as in-tube SPME was developed by using an open-tubular fused-silica capillary column as the SPME device instead of the SPME fiber for use in HPLC.[7–15] The main advantage of in-tube SPME is good analytical performance combined with simplicity, low cost and does not need a special interface. The open-tubular GC columns can be commercially available and have many various stationary phases.[8–10,12,14–15] Various capillaries were examined for pesticides, aromatic compounds, phthalates, organiarsenic compounds and medicines, etc. However, the extraction efficiency of the in-tube SPME is generally lower than that can be obtained with the SPE method.[7–15] To obtain higher extraction efficiency and reproducibility is important for a trace analysis and we must select suitable extraction capillaries for target analytes. Then, we set it as the purpose to search a column with the same extraction efficiency as SPE, and to determine the optimum conditions.

In-tube SPME studies of seven golf course pesticides in aqueous solutions were carried out by using an open-tubular column and a porous-layer open-tabular (PLOT) column. Since a PLOT capillary showed the better extraction efficiency for seven golf course pesticides, an in-tube SPME technique based on this capillary was studied in detail, and finally was coupled with Liquid Chromatography for the determination of seven golf course pesticides in aqueous samples.

MATERIALS AND METHODS

Reagent and Chemicals —— Methyl Sulfanilylcarbamate (Asulam), Bis (dimethylthiocarbamoyl) Disulfide (Thiuram), 3,5,6-Trichloro-2-pyridyloxyacetic acid (Triclopyr), (RS)-2-(4-Chloro-*o*-tolyloxy) propionic Acid (Mecoprop), Methyl (E)-(2-[6-cyanophenoxy] pyrimidin-4-yloxy) phenyl)-3-methoxyacrylate (Azoxystrobin), 1-(2-Methylcyclohexyl)-3-phenylurea (Siduron), Methyl 3-Chloro-5-(4,6-dimethoxypyrimidin-2-yl-carbomoylsulfamoyl)-1-methylpyrazole-4-carboxylate (Halosulfuronmethyl), 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-trifluoromethyl-2-pyridylsulfonyl) urea (Flazasulfuron) were obtained as mixture standards from Kanto Kagaku (Tokyo, Japan).The concentrations of these compounds were 10 ug ml^{-1} each other. Ethylenediamine-N,N,N′,N′-tetraacetic acid disodium salt dehydrate (EDTA), potassium dihydrogenphosphate and HPLC grade acetonitrile were obtained from Wako Chemicals (Osaka, Japan). Pure water was purified with a Milli-Q Gradient system (Japan Millipore, Tokyo, Japan).

Sample Preparation —— Water samples were collected in 250 ml glass bottles (Shibata Scientific, Tokyo, Japan) and filtered through 47 mm diameter, 1 um glass fiber filters (Toyo roshi, Tokyo, Japan). A 200 ml aliquot of the sample was prepared by adding 0.074 g of EDTA•2Na and pH-adjustment to 3.0 with 0.1 M phosphoric acid.

Apparatus ——

Liquid Chromatography: An LC10 series Liquid chromatograph (Shimadzu, Kyoto, Japan) consisted of a pump, an UV/visible (UV/Vis) detector, a column oven and a degassor. The separation was carried out by using Wakosil-Agri-9 column (4.6 i.d. × 250 mm, 5 μm particle size; Wako Chemicals). The mobile phase was prepared by mixing acetonitrile and buffer solution at the ratio of 45/55 (v/v). The buffer solution contained 6.8 g l^{-1} Potassium Dihydrogenphosphate and 0.18 g l^{-1} EDTA•2Na and adjusted to pH 3.7 with 0.1 M H_3PO_4. Flow rate was 1.0 ml min^{-1}. The ultraviolet detection was carried out at 230 nm, and the column temperature was controlled at 40°C. Data acquisition and processing was performed using C-R7A (Shimadzu, Kyoto, Japan). A Model 7125 valve (Reodyne, Cotati, CA, U.S.A.) was used as a syringe loading sample injector.

In-Tube Solid-Phase Microextraction: A GC capillary, Supel-Q PLOT (porous divinylbenzene polymer; 0.32 mm i.d. × 30 m) (Q-PLOT) was obtained from SPELCO (Bellefonte, PA, U.S.A.). A GC capillary, Inert Cap 5MS/Sil (0.32 mm i.d. × 30 m) (5MS) was obtained from GL Sciences (Tokyo, Japan). They are cut to 60 cm length and used as the in-tube SPME devices. The capillary was replaced

with a sample loop of the valve and connected with a polyether ether ketone (PEEK) tubing (0.33 i.d. × 20 mm; GL science),[7] a stainless nut, and a stainless ferrule. The sample volume was 1 ml; it was preconcentrated by in-tube SPME. A microsyringe pump KDS100 (KD Scientific, New Hope, PA, U.S.A.).equipped with 1005LTN gastight syringe (HAMILTON, Nevada, U.S.A.) was employed to sequentially pump the sample solution through the capillary in the valve. The capillary was washed with 1 ml pure water of a microsyringe and the valve was switched to the inject position, the extracted analytes were desorbed from the capillary with the mobile phase, and transported to a separation column.

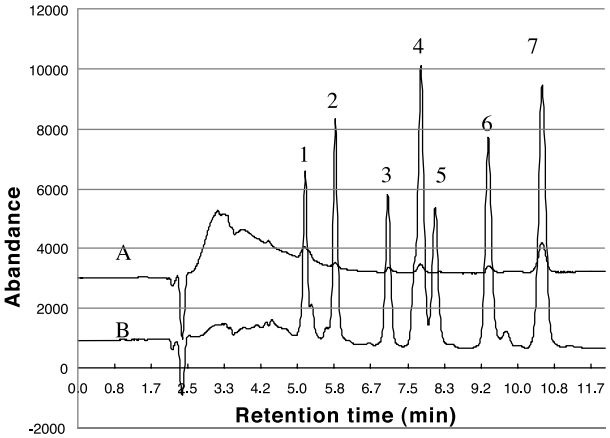

Fig. 1. The Typical Chromatograms of 7 Golf Course Pesticides of 10 ug l^{-1} by In-Tube SPME

A: Inert Cap 5MS/Sil and B: Supel-Q PLOT as extraction materials. Peaks: 1, Triclopyr 2, Mecoprop 3, Frazasulfuron 4, Thiuram 5, Halosulfuron methyl 6, Siduron 7, Azoxystrobin.

RESULTS AND DISCUSSION

Golf Course Pesticides

The guideline value of golf course pesticides was revised in the 2001 fiscal year and was established for 45 pesticides. Among the pesticides which cannot be measured by GCMS, nine highly polar or thermally labile pesticides which are presumed to be simultaneously extractive were set as the targets of the examination. Since golf course pesticides increased from 35 to 45 sorts, we studied about pesticides (Frazasulfuron, Halosulfuron methyl, Siduron, Azoxystrobin) added newly. Oxine-copper differed in that a peak overlaps with others in part, and a measurement wavelength was different from others. Moreover, asulam has a low recovery and reproducibility. So we removed them from the targets of simultaneous analysis. Then we studied seven pesticides.

Optimization of the Chromatographic System

A lot of reports on conventional in-tube SPME indicated that multiple draw/eject cycles are required to achieve right extraction efficiency.[7–13,15] The multiple draw/eject cycles were automatically carried out by using a programmable special autosampler.[7–16] We used a simple microsyringe pump equipped with a gastight syringe for sequentially pumping the sample solution through the capillary. It is able to control injection speed, injection time, and injection volume easily. The sample solution containing seven pesticides in aqueous EDTA solution (pH 3.0) was pumped through the extraction capillary by the microsyringe pump at the flow-rate of 100 μl min^{-1} for 10 min with the 7125 valve in the load position. After washing purewater (1 ml), the valve was switched to the inject position and the extracted pesticides were desorbed from the capillary with mobile phase flow and transported to the LC column. After 5 min, the valve was switched to the load position for the next analysis.

For selecting suitable extraction capillaries for target analytes, we investigated the reference of in-tube SPME. In pesticide studies, for carbamates, the extraction efficiency of Omegawax 250 column showed 9.1–37.4%.[14] Moreover, for phenylurea pesticides, those of polypyrrole (PPY), poly-N-methyl pyrrole (PMPY) and Supel-Q PLOT showed 24.3–33.6 and 18.2–31.9%, respectively.[15] A PLOT column has a porous layer on the inner wall. Since porous layer has a larger surface area, it seems that extraction efficiency may be high. Two different capillaries (Q-PLOT, 5MS) were employed to compare their efficiencies for the extraction of seven golf course pesticides from aqueous solution. Figure 1 shows the typical chromatograms of seven pesticides by in-tube SPME using a conventional open-tubular capillary column, 5MS, and a Q-PLOT column as extraction materials. As shown Fig. 1, of all the capillaries studied, a Q-PLOT column gave the best extraction efficiency, as compared to a 5MS column for almost all pesticides without asulam. It is clear that SPME with a Q-PLOT column exhibits a powerful ability for pre-concentration of seven pesticides in water samples. A Q-PLOT GC capillary column was used for further study.

The effect of the pH of the sample on the extraction of pesticides by in-tube SPME was examined

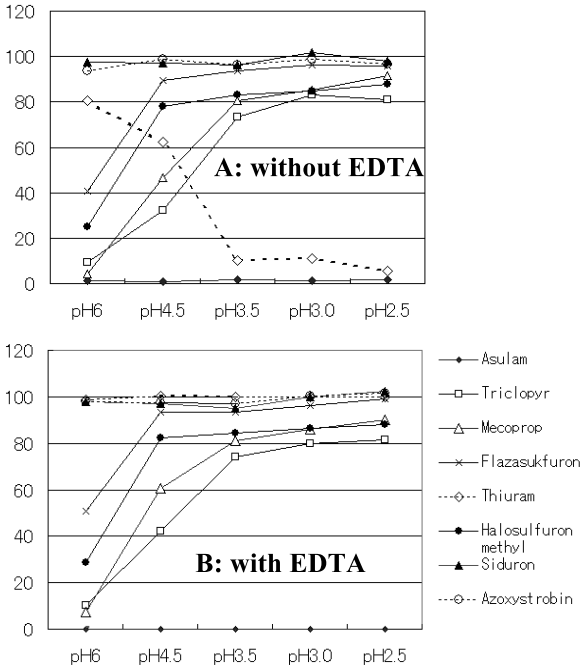

Fig. 2. The Effect of pH of the Sample on the Extraction of Pesticides by In-Tube SPME

A: sample without EDTA B: sample with EDTA. Concentrations of EDTA in the tested solutions were all the same (0.01%).

Table 1. Correlation between the Injection Speed and the Recovery

Pesticide	injection speed (ul min^{-1})			
	50	100	150	200
				(%)
Triclopyr	81.0	83.1	82.4	79.0
Mecoprop	96.0	95.2	82.2	79.1
Flazasukfuron	93.0	89.3	92.7	87.0
Thiuram	95.9	97.0	98.9	97.4
Halosulfuron methyl	98.2	91.1	87.2	81.8
Siduron	95.3	92.4	89.4	85.9
Azoxystrobin	93.6	91.6	91.8	89.8

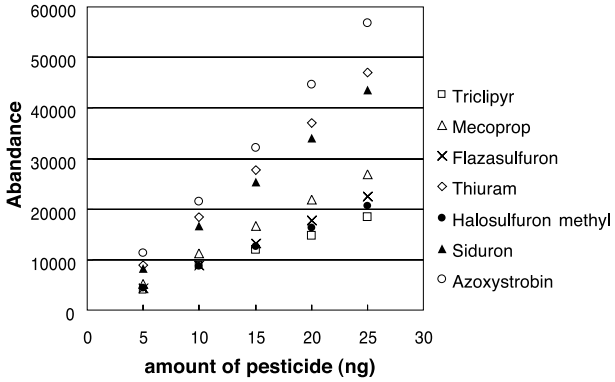

Fig. 3. The Linearity of Large Volume Injection by In-Tube SPME

They were investigated using 1 ml volume of sample solution including seven golf course pesticides.

by using pH 2.5, 3.0, 3.5, 4.5, and 6.0 solution. According to the study of Fujimoto, the case to which the recovery of Thiuram fell extremely at the measurement of river water and improved by adding EDTA was confirmed. Therefore, addition of EDTA was studied. The amount of adding EDTA was set up similarly to that of analysis for the golf course pesticides using SPE.[17] The effect of adding 0.01% EDTA was examined. As shown Fig. 2A, when EDTA was not added to a sample, six pesticides without thiuram showed good recovery at the time of 3.0 or more pH conditions. The recovery of thiuram decreased to 10% at the time of 3.5 or less pH conditions. As shown Fig. 2B, when EDTA was added to a sample, the recovery of thiuram was improved to 80% at the time of 3.5 or less pH conditions and the seven pesticides have been measured with a good recovery rate. A sample was prepared by adding EDTA•2Na (0.01%) and adjustment to pH 3.0 with 0.1 M phosphoric acid for further study.

Relationship between the injection speed and the recovery was established. A 1 ml aliquot of standard solution at 10 ng ml^{-1} was extracted by in-tube SPME at the injection speed of 50, 100, 150, 200 ul min^{-1}. As shown in Table 1, the best recovery was at the 50 ul min^{-1} injection speed. The recoveries of mecoprop, halosulfron-methyl, and siduron decreased as injection speed became fast. But there were slight differences of recovery. In fact too late loading speed is unsuitable for analyzing many samples. So we selected 100 ul min^{-1} injection speed.

Relationship between the linearity and the injection amount was established. The linearity of large volume injection by in-tube SPME was investigated by using a 1 ml aliquot of sample solution including eight golf course pesticides. The amounts of each pesticide in a sample solution were varied from 4 to 25 ng. As shown in Fig. 3, good linearity was acquired with a correlation coefficient of 0.999 for each over a range of 5–25 ng without any asulam.

Finally, the recoveries of seven golf course pesticides by in-tube SPME under optimal conditions were estimated from the amounts of analytes extracted in the stationary phase. As shown in Table 2, the recoveries of the seven analytes were in the range

Table 2. Recovery, LOD, and LOQ

	5MS	Q-PLOT		Q-PLOT	
	Recovery	Recovery	RSD	LOD	LOQ
	(%) ($n = 1$)	(%)	($n = 5$) (%)	(ug l^{-1})	(ug l^{-1})
Triclopyr	0.0	79.9	2.6	0.5	2.6
Mecoprop	2.2	86.1	2.8	0.7	2.8
Flazasulfuron	3.7	96.3	0.9	0.8	0.9
Thiuram	4.7	100.0	1.1	0.7	1.1
Halosulfuron methyl	0.7	86.5	4.1	0.6	4.1
Siduron	3.6	99.8	3.6	1.2	3.6
Azoxystrobin	12.0	100.5	1.4	0.8	1.4

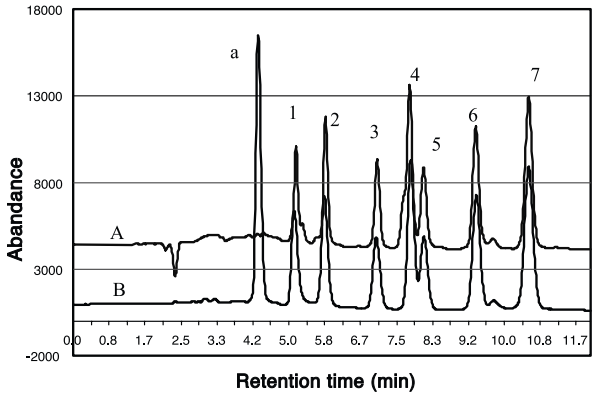

Fig. 4. The Typical Chromatograms of 8 Golf Course Pesticides of 10 ug l^{-1}

A: in-tube SPME and B: Direct (Autosampler) Peaks: a, Asulam 1, Triclopyr 2, Mecoprop 3, Frazasulfuron 4, Thiuram 5, Halosulfuron methyl 6, Siduron 7, Azoxystrobin.

from 79.9 to 100%. These results showed much the same recovery in comparison with those of the SPE method.[18] The chromatogram in Fig. 4 was obtained under the following conditions: 10 ul volume of a 1 mg l^{-1} sample solution dissolved in acetonitrile was introduced from an auto sampler and 1 ml volume of a 10 ug l^{-1} sample solution diluted in water was introduced by in-tube SPME.

Linearity and Recovery

To test the linearity of the calibration curves, various amounts of pesticides in the range 1–50 ng ml^{-1} were analyzed. The linearity was very good for all pesticides with correlation coefficients (r^2) higher than 0.999. The sensitivity of this analytical procedure was evaluated in terms of the limit of detection (LOD) calculated using S/N = 3 and the limit of quantification (LOQ) defined as tenfold the standard deviation with a spiked real sample such as river water. For the calculation of LOD, a water sample from the Kashima river, in which no traces of these pesticides were found, was spiked with 10 ng ml^{-1} of each pesticide. The LOQ was calculated from the results of repeatability. As shown in Table 2, the LOD and LOQ of each pesticide by this method were in the range 0.5–1.2 and 0.9–4.1 ng ml^{-1}, respectively. The accuracy of these quantitative results was in the range 1–5%.

We conducted the recovery test from river waters under the optimum conditions. The spiked-level of pesticides is 10 ug l^{-1}. As shown in Table 3, the recoveries of the spiked analytes ranged from 70.8 to 96.5%, and reproducibilities of this method were found to be relative standard deviation (RSD) 0.9–7.3% for five replicates. At the recovery test from waste water, the recoveries of the spiked analytes ranged from 64.4 to 97.7%, and reproducibilities of this method were found to be RSD 1.4–4.1% for five replicates.

We checked the decline of the extraction efficiency of the column. The extraction performance was only slightly decreased after the sequential sample extraction of more than 100 times. Since the column for in-tube SPME can be used about 100 times, the running cost is very low.

Seven golf course pesticides of several water samples in Chiba City were investigated by using the developed method in the summer and autumn of 2004. As the result of the autumn survey, mecoprop was found at 0.7 ng ml^{-1} from one golf course runoff. But the seven golf course pesticides were not detected from 5 river waters and 5 outflow waters of other golf courses. Although part of them was detected only by low concentration, also in order to check whether use of agricultural chemicals is managed appropriately, we need to continue this investigation further.

The new in-tube SPME method presented the

Table 3. Recovery of Seven Pesticides from Spiked Water

	river 1		river 2		outflow	
	Recovery	(RSD, %)	Recovery	(RSD, %)	Recovery	(RSD, %)
						($n = 5$)
Triclopyr	70.8	2.9	72.6	2.6	64.4	2.6
Mecoprop	88.2	3.6	80.9	2.8	72.8	4.1
Flazasulfuron	95.9	1.7	92.7	0.9	92.6	1.8
Thiuram	92.0	2.7	94.7	1.1	94.9	1.4
Halosulfuron methyl	81.0	7.3	83.0	4.1	81.8	3.4
Siduron	95.0	2.1	93.1	3.6	93.7	3.3
Azoxystrobin	95.8	1.5	96.5	1.4	97.7	2.0

following advantages over the conventional in-tube SPME HPLC. Since a PLOT capillary showed the better extraction efficiency for seven golf course pesticides, our method has realized the same recovery as a solid phase extraction method, and is superior to that of other researches. It is a semi-automated method, requiring no sample manipulation between the extraction and the HPLC analysis, therefore offering a high efficiency and precision. This method has a reasonably low LOD for UV analysis of the golf course pesticides studied. Since the column for in-tube SPME can be used about 100 times, the running cost is very low. This method requires no extracting solvent, and is eco-friendly.

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