FULL PAPER
THE ANALYST
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Automated on-line in-tube solid-phase microextraction followed by liquid chromatography/electrospray ionization-mass spectrometry for the determination of chlorinated phenoxy acid herbicides in environmental waters

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Received 9th January 2001, Accepted 19th March 2001
First published as an Advance Article on the web 19th April 2001

A method for the determination of six chlorinated phenoxy acid herbicides in river water was developed using in-tube solid-phase microextraction (SPME) followed by liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS). In-tube SPME is an extraction technique for organic compounds in aqueous samples, in which analytes are extracted from a sample directly into an open tubular capillary by repeated draw/eject cycles of the sample solution. Simple mass spectra with strong signals corresponding to $[M–H]^-$ and $[M–RCOOH]^-$ were observed for all herbicides tested in this study. The best separation of these compounds was obtained with a C_{18} column using linear gradient elution with a mobile phase of acetonitrile–water containing 5 mmol l^{-1} dibutylamine acetate (DBA). To optimize the extraction of herbicides, several in-tube SPME parameters were examined. The optimum extraction conditions were 25 draw/eject cycles of 30 μl of sample in 0.2% formic acid (pH 2) at a flow rate of 200 μl min^{-1} using a DB-WAX capillary. The herbicides extracted by the capillary were easily desorbed by 10 μl acetonitrile. Using in-tube SPME-LC/ESI-MS with time-scheduled selected ion monitoring, the calibration curves of herbicides were linear in the range 0.05–50 ng ml^{-1} with correlation coefficients above 0.999. This method was successfully applied to the analysis of river water samples without interference peaks. The limit of quantification was in the range 0.02–0.06 ng ml^{-1} and the limit of detection (S/N = 3) was in the range 0.005–0.03 ng ml^{-1}. The repeatability and reproducibility were in the range 2.5–4.1% and 6.2–9.1%, respectively.

Introduction

Chlorinated phenoxy acid herbicides are a major class of herbicides used in Japan and account for the majority of pesticides applied to farmland in the USA for weed control in row crops, such as corn and soybeans. They are inexpensive and normally applied.[1] They generally have low mammalian toxicity, but impurities and high dosages may cause teratogenic effects in rodents. Furthermore, several recent studies have demonstrated the occurrence of chlorinated phenoxy acid herbicide metabolites in surface water and groundwater.[2–4] Toxicological studies of metabolites are underway to assess whether the presence of these compounds is important to the total burden of pesticides in surface water and groundwater. (2,4-Dichlorophenoxy) acetic acid (2,4-D) and triclopyr have been regulated in drinking water in Japan in recent years.[5] (4-Chloro-2-methylphenoxy) acetic acid (MCPA) has been regulated in Japan for some time. The regulation value is in the range 5–30 ng ml^{-1} in drinking water and river water.

The methods used to determine chlorinated phenoxy acid herbicides, including EPA Methods 8150[6] and 8151,[7] involve gas chromatography (GC) with electron capture detection (ECD)[8–11] and mass spectrometric detection.[12–15] However, for GC methods, a prior derivatization step using diazomethane, alkyl and haloalkyl halides (*e.g.* methyl iodide, benzyl bromide and pentafluorobenzyl bromide) is necessary because of the low volatility and high polarity of chlorinated phenoxy acid herbicides. Furthermore, an extraction step, including liquid–liquid extraction,[15] solid-phase extraction[14] and solid-phase microextraction,[16] is necessary because derivatization cannot be performed in water solution. Only methylation of chlorinated phenoxy acid herbicides directly in water has been reported.[17] Therefore, new techniques to avoid extraction and derivatization in the analysis of these herbicides are desirable to increase the simplicity and reduce the analysis time.

Alternative methods based on liquid chromatography (LC) have been proposed.[18,19] These procedures do not require extraction or derivatization and employ a conventional reversed phase C_{18} column to separate the compounds. However, few of these compounds lack a strong chromophore above 220 nm, and a matrix peak attributed to fulvic and humic substances present in river water appears at the beginning of the chromatogram, co-eluting with the more polar of these compounds.[19] Another major limitation of LC-UV methods, such as the official method in Japan for pesticide analysis in environmental water, is the lack of mass spectrometric confirmation. Therefore, for more exact identification of target compounds, mass spectrometry (MS), which can obtain structural information, is the method of choice. Several techniques for the LC-MS method of the analysis of chlorinated phenoxy acid herbicides, including particle beam (PB),[20,21] thermospray ionization (TSI)[20,22] and electrospray ionization (ESI),[23,24] have been reported. LC/ESI-MS is well suited to the determination of chlorinated phenoxy acid herbicides because these compounds are efficiently ionized under electrospray conditions. Furthermore, to improve the sensitivity of pesticide determination in aqueous solution, solid-

 DOI: 10.1039/b100380l

phase extraction (SPE)[25–27] and solid-phase microextraction (SPME)[28–30] are often applied as preconcentration methods that can be used off-line and on-line with LC/ESI-MS. On-line techniques are very useful, because all analytes extracted on the sorbent can be transferred into the analytical column and the amount of solvent and sample can be reduced. However, special equipment is necessary for on-line SPE. On the other hand, on-line SPME, recently developed by Zhang *et al.*,[31] is an extraction technique using a fused-silica fibre that is coated on the outside with an appropriate stationary phase. The method saves preparation time, solvent purchase and disposal costs, and can improve the detection limits. It has been used routinely in combination with GC and GC-MS,[31,32] and has recently been introduced for interfacing with LC[33,34] and LC-MS. However, to date, the applications of SPME-LC are all based on a manual device. Furthermore, the selectivity obtained for the analysis of very polar compounds is still poor because of a limited selection of commercially available fibre coatings.

In-tube SPME is a new variation of SPME that has recently been developed using GC capillary columns as the SPME device instead of the SPME fibre. In-tube SPME is suitable for automation, and automated sample handling procedures not only shorten the total analysis time but also usually provide better accuracy and precision relative to manual techniques. In-tube SPME can be easily coupled on-line with high performance liquid chromatography (HPLC) for the determination of less volatile and/or thermally labile compounds.[35–38] Furthermore, recently, conducting polymers, such as polypyrrole, have been applied for in-tube SPME for the analysis of catechin and caffeine in tea.[39]

In this study, an automated in-tube SPME method coupled with LC/ESI-MS was developed for the determination of six chlorinated phenoxy acid herbicides. This was facilitated by Agilent Technologies 1100 LC-MS system, as the standard autosampler for this system (1100 series autosampler) is ideally suited for in-tube SPME. A schematic diagram of the automated in-tube SPME-LC/ESI-MS system is illustrated in Fig. 1. In this technique, chlorinated phenoxy acid herbicides in aqueous samples are extracted directly from the sample into the internally coated stationary phase of a capillary. The capillary is placed between the injection loop and the injection needle of the autosampler. While the metering pump repeatedly draws and ejects sample from the vial, the analytes partition from the sample matrix into the stationary phase until equilibrium is reached. The extracted analytes are directly desorbed from the stationary phase by mobile phase flow or additional solvent, transported to the LC column, and then detected by the mass selective detector (MSD). The potential of the final method is demonstrated by its application to the analysis of river water.

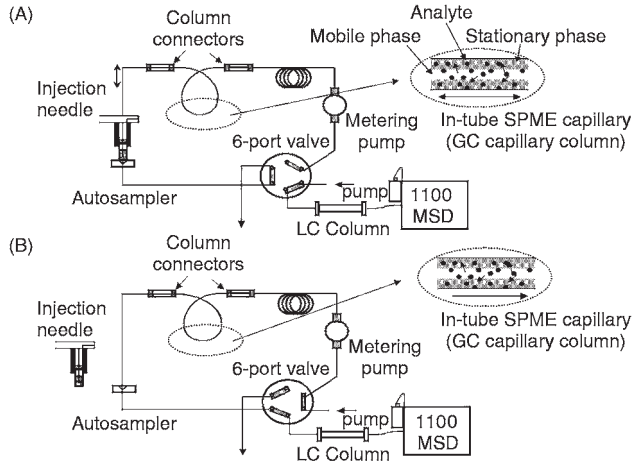

Fig. 1 Schematic diagram of in-tube SPME: (A) load position (extraction phase); (B) injection position (desorption phase).

Experimental

Chemicals

(4-Chloro-2-methylphenoxy) acetic acid (MCPA), (2,4-dichlorophenoxy) acetic acid (2,4-D), 2-(2,4-dichlorophenoxy) propionic acid (Dichlorprop), (2,4,5-trichlorophenoxy) acetic acid (2,4,5-T), 4-(2,4-dichlorophenoxy) butyric acid (2,4-DB) and 3-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP) were obtained as individual standards from Hayashi Pure Chemicals (Osaka, Japan). The purity of these compounds was higher than 99%. Stock solutions containing all chlorinated phenoxy acid herbicides at 1 mg ml^{-1} were prepared in methanol, stored in the dark at 4 °C and diluted to the desired concentrations prior to use. Ammonium acetate, formic acid, dibutylamine acetate (DBA) and acetonitrile were obtained from Wako Chemicals (Osaka, Japan). All other chemicals were of reagent grade. Pure water was purified with a Milli-Q system (Millipore, Tokyo, Japan).

Sample preparation

River water was collected in 100 ml glass bottles (Shibata Scientific, Tokyo, Japan) and filtered through 13 mm diameter, 0.2 μm nylon syringe filters (Toso, Tokyo, Japan). A 900 μl aliquot of the sample was transferred to a 2 ml glass vial (Agilent Technologies, Palo Alto, CA, USA) and 20 mg ml^{-1} formic acid (100 μl) was added to the vial. Calibration curves of the six herbicides were constructed by in-tube SPME-LC/ESI-MS using time-scheduled selected ion monitoring (SIM) of a sample spiked with 0.05–50 ng ml^{-1} of the six chlorinated phenoxy acid herbicides.

Apparatus

Liquid chromatography-mass spectrometry

An Agilent 1100 series liquid chromatograph (Agilent Technologies, Waldborn, Germany), consisting of a vacuum solvent degassing unit, a binary high pressure gradient pump, an autosampler and a column thermostat, was used for LC-MS analysis. Furthermore, a model Agilent 1100 series diode array detector (Agilent Technologies, Waldborn, Germany) was connected on-line with the MSD. LC separation was performed on a 150 × 2.1 mm id column packed with 5 μm Inertsil ODS3 (GL Science, Tokyo, Japan) using linear gradient elution for 15 min with a mobile phase of acetonitrile–water containing 5 mmol l^{-1} DBA (from 30 + 70 to 85 + 15 v/v). The flow rate was 200 μl min^{-1}. The sample volume was 1 ml, it was preconcentrated by on-line in-tube SPME using an autosampler, and all analytes were transferred to the MS system.

An Agilent 1100 series MSD single quadrupole instrument, equipped with orthogonal spray-ESI (Agilent Technologies, Palo Alto, CA, USA), was used. All MS optimization steps were carried out in the ESI negative ion mode using the analytical column. Nitrogen as nebulizing and drying gas (350 °C) was generated from compressed air using a Whatman model 75-72 nitrogen generator (Whatman, Haverhill, MA, USA). The nebulizing gas pressure was set at 60 psi and the drying gas was held at 12 l min^{-1}. The drying gas temperature was kept at 350 °C and the fragmentor voltage for in-source fragmentation was set at 100 V. Furthermore, skimmer 1, 2 and entrance lens voltages in the ion source of MSD were automatically optimized using a calibration standard (Agilent Technologies, Palo Alto, CA, USA) and set at 23 , 47 and 57 V, respectively. Mass spectra were acquired over the scan range *m/z* 100–500 atomic mass units (u) using a step size of 0.1 u and a scan speed of 0.5 scan s^{-1}. Quantitative analysis was carried out using the

SIM mode of base ion peaks at *m/z* 199 (MCPA), 219 (2,4-D), 233 (Dichlorprop), 255 (2,4,5-T), 161 (2,4-DB) and 267 (2,4,5-TP) with a dwell time of 250–500 ms. To verify the presence of target analytes in river water, the halogen isotopic ion of all target analytes was monitored.

In-tube solid-phase microextraction

A schematic diagram of the in-tube SPME-LC/ESI-MS system is illustrated in Fig. 1. Briefly, a GC capillary (60 cm x 0.25 mm id, 1.0 μm film thickness) coated by a DB-WAX stationary phase (J&W Scientific, Folsom, CA, USA) was used as the in-tube SPME device and placed between the injection loop and injection needle of the autosampler. The injection loop was retained in the system to avoid fouling of the metering pump. Capillary connection was facilitated by the use of a 2.5 cm sleeve of 1/16 in polyether ether ketone (PEEK) tubing (GL Science, Tokyo, Japan) at each end of the capillary.[38] PEEK tubing of 0.33 mm id was found to be suitable to accommodate the capillary. Normal 1/16 in stainless steel nuts, ferrules and union (GL Science, Tokyo, Japan) were then used to complete the connection. The autosampler software was programmed to control the in-tube SPME extraction, desorption and injection. Two millilitre vials filled with 1 ml of sample were set into the autosampler programmed to control the in-tube SPME. In addition, 1.5 ml each of methanol and pure water in 2 ml vials were set into the autosampler. In a first step, the capillary column was washed and conditioned by two repeated draw/eject cycles (30 μl each) of methanol and pure water in this order prior to extraction. The extraction of chlorinated phenoxy acid herbicides into the capillary was performed using 25 repeated draw/eject cycles of 30 μl of sample at a flow rate of 200 μl min^{-1} in the vial with the six-port valve in the LOAD position. After drawing acetonitrile (10 μl), the six-port valve was switched to the INJECT position and the extracted herbicides were desorbed from the capillary with acetonitrile and mobile phase flow and transported to the LC column. For the maintenance of the GC capillary as the in-tube SPME device after fifty samples, the GC capillary should be rinsed with 100 μl acetonitrile and removed from the instrument followed by purging of the remaining solvent in the capillary using dry nitrogen gas. The bonded and cross-linked stationary phase is not damaged by the injection of organic solvent, but inorganic acid (HCl, H_2SO_4, H_3PO_4, HNO_3, *etc*.) and base (KOH, NaOH, *etc*.) will lead to damage. Therefore, organic solvent should be injected into the capillary for the rinse and inorganic acid should not be used as mobile phase additive. For the storage of the capillary, each end of the capillary should be plugged using a stainless steel union in order to avoid oxidation of the stationary phase by air.

Results and discussion

Liquid chromatography/electrospray ionization-mass spectrometry optimization

Influence of the buffer on the sensitivity and retention of the herbicides A good separation of the acidic compounds is feasible using a C_{18} silica column with an acidic buffer[23] or an ion-pairing buffer.[38] However, for the case in which an acidic buffer was used, the post-column addition of neutralization buffer was required in order to form ions in solution and to facilitate charging of droplets. An equimolar amount of triethylamine was therefore added to the formic acid mobile phase at a flow rate of 0.1 ml min^{-1} by the tee union installed between the analytical column and the ion source. For the investigation of the retention behaviour of all herbicides, a standard mixture of the six chlorinated phenoxy acid herbicides at 1000 ng ml^{-1} was analysed in the SIM mode using 10 mmol l^{-1} ammonium acetate, 0.1% formic acid and 5 mmol l^{-1} DBA as the mobile phase modifiers. For the separation of all herbicides, the best results were obtained with 0.1% formic acid and 5 mmol l^{-1} DBA. However, the response factor was at least twice as sensitive for all herbicides when 5 mmol l^{-1} DBA rather than 0.1% formic acid was used with 0.1% triethylamine as the post-column additive. DBA (5 mmol l^{-1}) was therefore used for the separation of all herbicides by LC/ESI-MS in the negative ion mode. A typical total ion chromatogram of the standard mixture of six herbicides at 1 μg ml^{-1}, obtained under full scan mode, is shown in Fig. 6 (see later).

Evaluation of ESI performance and mass spectral information The mobile phase containing 5 mmol l^{-1} DBA as a volatile ion-pairing reagent is directly compatible with ESI-MS. However, the use of the ion-pairing reagent will cause analyte suppression and a high chemical background if the ESI-MS condition is not optimized, because the ion-pairing reagent forms a complex with target analytes.[40] The main operating parameters which have an impact on the performance of ESI are the fragmentor voltage (capillary exit voltage), the nebulizer gas pressure and the drying gas flow rate. It has been reported[40,41] that the intensity of the analyte does not show a large variation when the drying gas flow rate is varied from 4 to 13 l min^{-1}. For the nebulizer gas pressure, when the volatile ion-pairing reagent is used, a higher value ensures the best sensitivity for the analytes because it can break the 'neutral' ion-pairs of the analytes and improve the suppression of the intensity of the analytes.[41] These parameters were maintained at 12 l min^{-1} and 60 psi.

The fragmentor voltage is applied to the exit of the capillary and affects the transmission and fragmentation of sample ions. In general, the higher the fragmentor voltage (which aids the transfer of ions in the relatively high pressure region between the exit of the capillary and the skimmer) the greater the amount of fragmentation that will occur. In compounds that do not fragment readily, higher fragmentor voltages often result in better ion transmission. Thus, at higher voltage values, maximum structural information and sensitivity will be obtained. However, the optimum fragmentor voltage is compound dependent, and an accurate evaluation of the fragmentor voltage for the compounds studied in this work was performed using $[M–H]^-$ and $[M–RCOOH]^-$ ions in the scan mode. In Fig. 2, the intensities of the $[M–H]^-$ and $[M–RCOOH]^-$ ions for all herbicides are shown as a function of the fragmentor voltage.

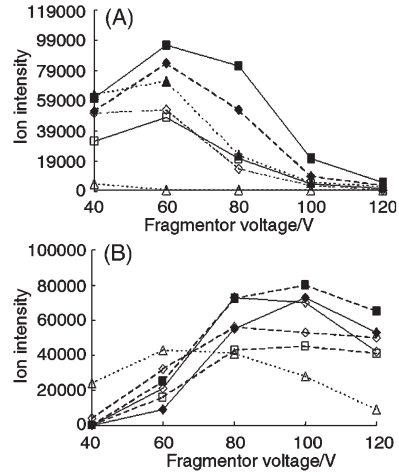

Fig. 2 Dependence of ionization and fragmentation on fragmentor voltage for chlorinated phenoxy acid herbicides. (A) $[M–H]^-$: ■, MCPA; ◆, 2,4-D; ▲, Dichlorprop; □, 2,4,5-T; Δ, 2,4-DB; ◇, 2,4,5-TP. (B) $[M–RCOOH]^-$: ■, MCPA; ◆, 2,4-D; ▲, Dichlorprop; □, 2,4,5-T; Δ, 2,4-DB; ◇, 2,4,5-TP.

For all herbicides except 2,4-DB, which exhibited [M–$(CH_2)_3COOH]^-$ as the base peak, $[M–H]^-$ ions were observed as the base peak at less than 60 V and presented maximum sensitivities. At higher fragmentor voltage, the intensities of $[M–H]^-$ ions decreased and the intensities of $[M–RCOOH]^-$ ions increased. Other ions observed in the mass spectra of all herbicides were isotopic ions which derived from the chlorine element and these ions were of maximum abundance for 2,3,5-T and 2,4,5-TP. The fragmentor voltage for all herbicides was set at 60 V and Table 1 shows the typical mass spectra of the six chlorinated phenoxy acid herbicides at 60 V.

Optimization of in-tube solid-phase microextraction The extraction efficiency in SPME can be evaluated by the determination of the amount of analytes extracted by the stationary phase. For the typical stationary phase that extracts analytes based on absorption, the amount of analytes extracted can be expressed as:

$$n_A = K_A V_f V_s C_A^0 / (K_A V_f + V_s) \quad (1)$$

where n_A is the amount of analyte A extracted by the stationary phase at equilibrium, V_s and V_f are the volumes of the sample solution and stationary phase, respectively, C_A^0 is the initial concentration of the analyte in the sample and K_A is the partition coefficient.

However, it is difficult to use the above equation to obtain n_A because some of the terms, such as K_A and V_f, are difficult to measure. Fortunately, n_A can easily be obtained by SPME by experimental measurement using the following equation:

$$n_A = FA = (m/A_d)/A \quad (2)$$

where n_A is the amount of analyte extracted by SPME, F is the detector response factor, which can be calculated by comparing the amount of analyte (m) injected and the area count (A_d) obtained by liquid injection ($F = m/A_d$), and A is the response obtained by SPME. Therefore, the extraction efficiencies (recoveries) for the sample analyte can be evaluated by comparing the n_A values obtained by SPME experiments under the same extraction conditions.

To optimize the extraction of chlorinated phenoxy acid herbicides by in-tube SPME, several parameters, such as the stationary phase of the capillary, sample matrix, the number of draw/eject cycles and the desorption solvent, were investigated. In this study, a standard mixture of the six chlorinated phenoxy acid herbicides at 100 ng ml^{-1} was analysed using the SIM mode. The draw/eject volume was set at 30 μl because the total internal volume of the capillary was 29.4 μl.

In-tube SPME is an equilibrium technique based on the partitioning of the solute between the stationary phase and the aqueous sample. Therefore, the parameters which affect the partitioning of the analytes are the type of stationary phase and the film thickness. First of all, three different capillaries (DB-WAX, DB-50 and DB-1) were employed to compare their efficiencies for the extraction of chlorinated phenoxy acid herbicides from aqueous solution. As shown in Fig. 3, of all the capillaries studied, the relatively polar DB-WAX gave the best extraction efficiency as compared to the less polar DB-50 and DB-1 for all herbicides.

The effect of the film thickness on extraction is very important in SPME. It is expected from eqn. (2) that the amount of analyte extracted will increase with increasing film thickness, because an increase in the film thickness means an increase in the total stationary phase volume (V_f). In this study, three capillaries of different thicknesses (0.25, 0.5 and 1.0 μm) were evaluated. As a result, maximum extraction efficiencies for all herbicides were obtained using the capillary of 1.0 μm film thickness. A GC capillary (60 cm x 0.25 mm id, 1.0 μm film thickness) coated with a DB-WAX stationary phase was used for further study.

Another parameter which affects the partitioning of the analytes is the sample matrix. The solubility of the analytes in the sample matrix changes with the pH of the sample and with the modifier, such as the ion-pairing reagent. The effect of the sample matrix modifier on the extraction of herbicides by in-tube SPME was examined using 5 mmol l^{-1} DBA, 0.2% acetic acid and 0.2% formic acid solution. As shown in Fig. 4, 0.2% formic acid solution (pH 2) as sample matrix modifier was most effective for all herbicides.

To obtain the extraction–time profiles of herbicides by in-tube SPME, the number of draw/eject cycles was varied from 5 to 30. After 30 draw/eject cycles, equilibrium conditions were not obtained for the extraction of all herbicides (Fig. 5). However, peak broadening for MPCA, 2,4-D and Dichlorprop, was observed at 30 cycles. This peak broadening was considered to be caused by the broadening of the bandwidth of the analytes extracted into the capillary. Best overall results were obtained using 25 draw/eject cycles of 30 μl of sample.

Table 1 Relative intensities (RI) of the main ions formed in ESI-MS of chlorinated phenoxy acid herbicides: m/z (RI, %)

Herbicide	$[M–H]^-$	$[M–RCOOH]^-$	Molecular mass
MCPA	199 (100)	141 (12)	200
2,4-D	219 (100)	161 (28)	220
Dichlorprop	233 (100)	161 (32)	234
2,4,5-T	255 (100)[a]	197 (29)	254
2,4-DB	247 (3)	161 (100)	248
2,4,5-TP	269 (100)[a]	197 (62)	268

[a] Isotopic ion condition: fragmentor voltage, 60 V.

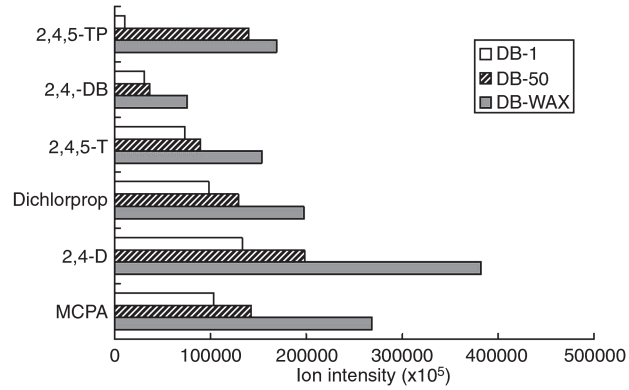

Fig. 3 Evaluation of three capillary columns for in-tube SPME-LC/ESI-MS of chlorinated phenoxy acid herbicides. Capillary column: 60 cm × 0.25 mm id, 0.25 μm film thickness. SPME conditions: herbicides, 100 ng ml^{-1}; sample pH 5; draw/eject cycle, 15; draw/eject volume, 25 μl; draw/eject rate, 200 μl min^{-1}; desorption, mobile phase.

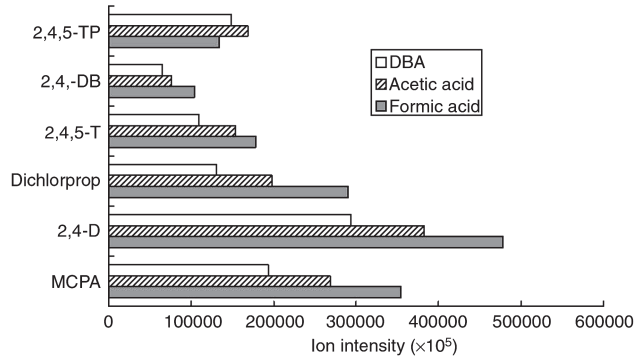

Fig. 4 Effect of sample modifier on the extraction efficiency of chlorinated phenoxy acid herbicides with DB-WAX capillary. Other conditions are the same as in Fig. 3.

The effect of desorption solvent on the desorption of herbicides from the capillary was examined using acetonitrile, methanol and the mobile phase. The maximum intensities of all analytes were obtained using 10 μl acetonitrile as desorption solvent. Above 10 μl, peak broadening was observed, although the intensities did not increase. This result indicates that most analytes extracted were trapped in the capillary tip. Furthermore, the excess desorption solvent caused band broadening of the analytes transferred into the LC column. Therefore, acetonitrile as the desorption solvent was set to 10 μl.

Finally, the recoveries of chlorinated phenoxy acid herbicides by in-tube SPME under optimal conditions were estimated from the amounts of analytes extracted in the stationary phase, calculated according to eqn. (2). As shown in Table 2, the recovery of analytes ranged from 23.9% to 30.7%. These results showed poor recovery in comparison with those of the SPE method.[14] Recent studies have reported that, for solutes with low octanol–water distribution coefficients ($k_{o/w} < 10000$), low recovery is observed using conventional SPME due to the high phase ratio (>10000).[42,43] The chlorinated phenoxy acid herbicides investigated in this study have low $k_{o/w}$ and these seem to be the cause of the low recoveries. However, in-tube SPME improved the absolute recoveries of herbicides in comparison with conventional SPME (typical recovery is less than 20%) by dynamic extraction using the autosampler.

The entire in-tube SPME extraction and desorption of chlorinated phenoxy acid herbicides was accomplished automatically in 10 min.

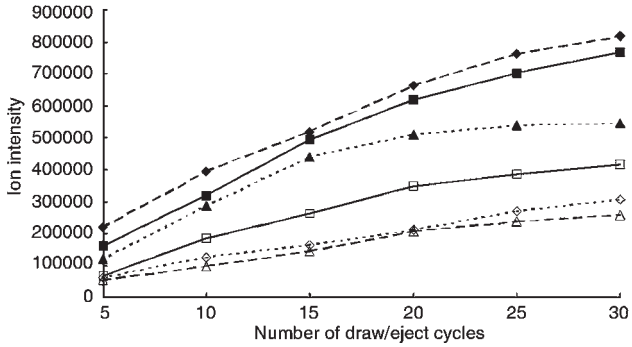

Fig. 5 Extraction–time profile of chlorinated phenoxy acid herbicides with DB-WAX capillary. Sample modifier, 0.2% formic acid. Other conditions are the same as in Fig. 3. ■, MCPA; ◆, 2,4-D; ▲, Dichlorprop; □, 2,4,5-T; Δ, 2,4-DB; ◇, 2,4,5-TP.

Table 2 Recovery of chlorinated phenoxy acid herbicides using in-tube SPME

	Peak intensity ($\times 10^5$)		
Herbicide	Direct injection[a]	In-tube SPME[b]	Recovery (%)
MCPA	5.52	14.35 (25.9 ng)[c]	25.9
2,4-D	7.65	18.32 (23.9 ng)	23.9
Dichlorprop	6.09	15.37 (25.2 ng)	25.2
2,4,5-T	6.33	16.52 (26.1 ng)	26.1
2,4-DB	3.94	10.83 (27.5 ng)	27.5
2,4,5-TP	4.09	12.54 (30.7 ng)	30.7

[a] A 10 μl aliquot of pure water spiked with 1000 ng ml^{-1} was directly injected.[b] A 1 ml aliquot of 0.1% formic acid solution at 100 ng ml^{-1} was extracted by in-tube SPME followed by desorption with 10 μl acetonitrile.[c] Extracted amounts were calculated in comparison with peak intensities in direct injection and in-tube SPME.

Linearity, detection limits and precision of the in-tube SPME-LC/ESI-MS system In order to achieve optimum sensitivity, all experiments were carried out under SIM conditions, and the $[M–H]^-$ ions were selected for all chlorinated phenoxy acid herbicides, except 2,4-DB, for which the $[M–(CH_2)_3COOH]^-$ ion was selected. To test the linearity of the calibration curves, various concentrations of herbicides in the range 0.05–50 ng ml^{-1} were analysed. As shown in Table 3, the linearity was very good for all herbicides 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 Kanzaki river water sample, in which no traces of these herbicides were found, was spiked with 0.05 ng l^{-1} of each chlorinated phenoxy acid herbicide. The LOQ was calculated from the results of repeatability. The LOD and LOQ of each herbicide by this method were in the range 0.005–0.03 ng ml^{-1} and 0.02–0.06 ng ml^{-1}, respectively. These LOD and LOQ values were much lower than those for LC-MS with thermospray[19] and particle beam,[19] and slightly lower than for on-line SPE-LC/ESI-MS.[22] The intra-day precision (repeatability) was calculated by analysing five river water samples spiked with 0.1 ng ml^{-1} during a working day. The inter-day precision (reproducibility) was calculated by analysing three samples spiked with 0.1 ng ml^{-1} over three working days. The repeatability and reproducibility of the method for all herbicides ranged from 2.5% to 9.1%. The quantitative results of all herbicides in the spiked river water at 0.1 ng ml^{-1} using external standards are shown in Table 3 and a SIM chromatogram of this sample is shown in Fig. 6. The accuracy of these quantitative results was in the range 10–20% and no significant interference peaks were observed.

Analysis of real river water samples and the robustness of the method The validity and robustness of the method were checked with real samples of river water. First of all, for method validation, 30 river water samples from the Yodo and Kanzaki rivers in Osaka were analysed. These results were contrasted with those obtained by conventional GC-MS[14] and HPLC methods[18] using SPE as the sample extraction technique. In seven samples, three herbicides were detected at the retention

Table 3 Sensitivity, linearity and precision of chlorinated phenoxy acid herbicides

		Sensitivity/ng ml^{-1}			Instrument precision (RSD, %)	
Herbicides	r^2	LOD[a]	LOQ[b]	Quantitative[c] results/ng ml^{-1}	Repeatability[d]	Reproducibility[e]
MCPA	0.9998	0.01	0.04	0.12	3.5	8.3
2,4-D	0.9996	0.005	0.02	0.09	2.5	9.1
Dichlorprop	0.9996	0.01	0.04	0.11	3.3	7.9
2,4,5-T	0.9998	0.02	0.02	0.12	2.6	8.7
2,4-DB	0.9994	0.03	0.06	0.11	4.1	7.3
2,4,5-TP	0.9992	0.02	0.04	0.08	3.7	6.2

[a] Limit of detection (LOD) was defined as S/N = 3 for the river water spiked with 0.05 ng ml^{-1}.[b] Limit of quantification (LOQ) was defined as ten standard deviations for five replicates of the river water sample spiked with 0.1 ng ml^{-1}.[c] Calculated for river water spiked at 0.1 ng ml^{-1}.[d] Repeatability was calculated by analysing five river water samples spiked with 0.1 ng ml^{-1} within 1 day.[e] Reproducibility was calculated by analysing three river water samples spiked with 0.1 ng ml^{-1} per day for 3 days.

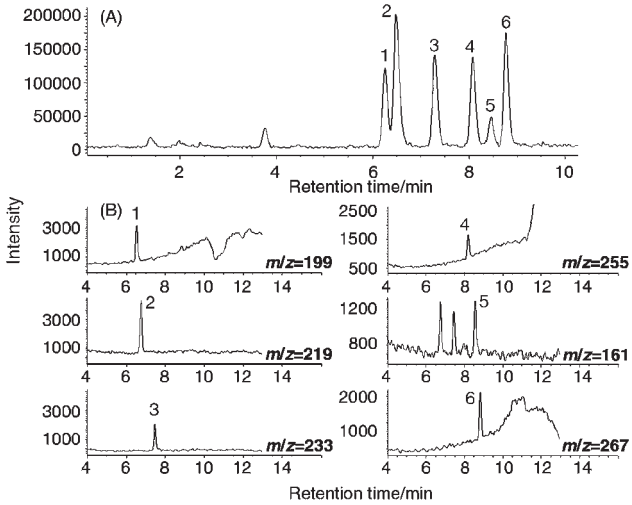

Fig. 6 Total ion chromatogram and SIM chromatogram of the six chlorinated phenoxy acid herbicides. (A) Total ion chromatogram was obtained using DBA as ion-pairing reagent. Eluent A (water) and B (acetonitrile) containing 5 mmol l^{-1} DBA. Linear gradient from 30% B/A to 85% B/A in 15 min, 15 min re-equilibration. (B) SIM chromatogram was obtained by in-tube SPME-LC/ESI-MS of river water spiked with 0.05 ng ml^{-1}. 1, MCPA; 2, 2,4-D; 3, Dichlorprop; 4, 2,4,5-T; 5, 2,4-DB; 6, 2,4,5-TP.

Table 4 Quantitative analysis of chlorinated phenoxy acid herbicides in river water samples (ng ml^{-1})

	LC/ESI-MS[a]		GC-MS[b]		HPLC-UV[b]	
Sample	MCPA	2,4-D	MCPA	2,4-D	MCPA	2,4-D
A	0.24	0.47	0.27	0.51	0.28	0.43
B	0.35	0.23	0.33	0.21	0.37	0.26
C	0.39	0.11	0.36	0.13	0.42	0.11
D	0.21	0.38	0.19	0.37	0.23	0.41

[a] In-tube SPME was used for sample preconcentration. Off-line SPE was used for sample preconcentration.

times of MCPA, 2,4-D and 2,4,5-T using a HPLC method. However, for three samples in which 2,4,5-T was detected, none of the peaks eluted at the same retention time using GC-MS and the current method. These samples were analysed under full scan acquisition using the current method and a large interference peak was detected at the same retention time as 2,4,5-T. Therefore, this peak was the reason why 2,4,5-T was detected by the HPLC method. Table 4 shows a comparison between the analyses of four river water samples by three methods using in-tube SPME and SPE preconcentration. As can be seen, good agreement was observed between the results obtained for MCPA and 2,4-D with the three detection techniques. These results indicate that in-tube SPME-LC/ESI-MS may be a valuable tool in the analysis of chlorinated phenoxy acid herbicides in river water samples.

To evaluate the robustness of the method, the system was used to analyse over 40 river water and standard samples. No maintenance was required (exchange the GC capillary, *etc*.) and no obstruction of the system was observed. This result indicates that the method is reliable and robust and is therefore applicable to routine analysis.

Conclusions

In-tube SPME is a fast sample preparation technique that can be operated automatically (autosampler). Furthermore, this technique does not require any special equipment (requires only a GC capillary column). The GC capillary column (length, 60 cm), used as the in-tube SPME device, can be employed for over 40 real samples. Therefore, because 50 capillaries can be made from one conventional GC capillary column (length, 30 m), over 1500 real samples can be analysed by one GC column. This indicates that the cost per sample is far cheaper than for on-line SPE. The recovery of in-tube SPME is lower than that of conventional SPE. However, the precision of this method is very good using the autosampler and is acceptable for routine analysis. Another advantage of the present system is that a mass spectrometric detector has been successfully coupled to ion-pair LC through ESI, which enhances the selectivity and identification capability of the method.

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