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Analysis of chlorophenoxy acid herbicides in water by large-volume on-line derivatization and gas chromatography–mass spectrometry

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Abstract

This work presents a modified method to analyze chlorophenoxy acid herbicides in water samples. The herbicides 2,4-D (2,4-dichlorophenoxyacetic acid), Silvex (2,4,5-trichlorophenoxypropionic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) were used to evaluate the method. The method involves extraction of samples by a graphitized carbon black cartridge, and on-line derivatization in the GC injection port using a large-volume (10–20 μl) direct sample introduction (DSI) device with tetraalkylammonium salts. The analytes were then identified and quantitated by ion-trap gas chromatography–mass spectrometry. The large-volume DSI injection-port derivatization technique provides sensitivity, fast and reproducible results for chlorophenoxy acid herbicides residues, to quantitation at 0.1 to 0.2 μg/l in 500-ml water samples. An enhanced characteristic mass chromatogram of molecular ions of butylated chlorophenoxy acid herbicides with a significant chlorine isotope pattern by electron impact ionization MS allows us to determine herbicides residues at trace levels in aqueous samples. Recovery of the herbicide residues in spiked various water samples ranged from 70 to 99% while RSDs ranged from 1 to 13%.

Keywords: Derivation, GC; Injection methods; Large-volume injection; Water analysis; Environmental analysis; Chlorophenoxy acids; Phenoxy acids; Pesticides

1. Introduction

Chlorophenoxy acids are an important group of herbicides widespread in agriculture, industrial weed-control and forestry. These herbicides are common in the surface water and groundwater near agricultural fields or golf courses [1–3]. Various solid-phase extraction (SPE) methods combined with high-performance liquid chromatography (HPLC) or gas chromatography–mass spectrometry (GC–MS) as identification and quantitation methods have been developed [1–8], and reviewed by Sherma [9].

To employ high-resolution GC as the determination step for phenoxy acid herbicides, derivatization is required to increase analyte volatility and improve chromatographic separation. Diazomethane is the most frequently used derivatization procedure for phenoxy acid analysis. However, the toxicity, carcinogenicity and explosiveness of this agent, means that alternative procedures must be evaluated. Thus, sulfuric acid with *n*-propanol or methanol, and boron trifluoride in methanol, *n*-butanol or 2-chloroethanol has been applied to esterify such acids [10]. Currently, the injection-port derivatization procedure with an ion-pair reagent has been successfully

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applied to determine ionic and carboxylate surfactants and their degradation products in water samples [11,12]. The procedure was initiated by reacting carboxylic acid or sulfonate groups with tetraalkylammonium (TAA) salts [i.e., tetrabutyl-ammonium hydrogensulfate, $N(Bu)_4^+HSO_4^-$] to form carboxylate ion pairs [$RCOO^-N(Bu)_4^+$] in solution. Upon introduction to a high-temperature (300°C) GC injection port, the carboxylic acid groups were transformed to their corresponding butyl esters [RCOOBu].

The large-volume sample introduction is an attractive method of improving detection sensitivity, and of preventing discrimination inside the syringe needle and injector liner from injecting a small volume of sample. Mol et al. have evaluated and reviewed the technique of inserting glass wool in the large dimensions of injector liners, and referred to this sample introduction method as ''solvent-split injection'' [13]. Application of a temperature-programmed injector with the direct sample introduction (DSI) device to large-volume injection in capillary GC for trace analysis has been described elsewhere [12,14].

This work presents the results of a modified method for rapid and quantitative determination of chlorophenoxy acid herbicide residues in aqueous samples. Sensitivity and precision were determined following optimization of the on-line derivatization approach using 2,4-D (2,4-dichlorophenoxyacetic acid), Silvex (2,4,5-trichlorophenoxypropionic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) as trial analytes in aqueous samples.

2. Experimental

2.1. Samples

Water samples were collected from a river that receives runoff or untreated wastewaters from suburban agricultural areas in Tao-Yuan County (Taiwan). Samples were collected at two sampling sites where the samples content had variable compositions [15], and can be represented as typical river samples in Taiwan. The samples were collected at a 0.5-m depth from mid-stream using pre-rinsed glass bottles. Three replicate 500-ml samples were collected and shipped to the laboratory in ice-packed containers. Upon arrival, the samples were immediately adjusted to pH 2–3 by adding concentrated HCl, and then stored at 4°C until analysis.

2.2. Chemicals and reagents

Unless stated otherwise, all high-purity chemicals and solvents were purchased from Aldrich (Milwaukee, WI, USA), Tedia (Fairfield, OH, USA) and Merck (Darmstadt, Germany), and were used without further purification. Reagent-grade tetrabutylammonium hydrogensulfate (TBA-HSO_4) and tetramethylammonium hydrogensulfate (TMA-HSO_4) were purchased from TCI (Tokyo Chemical Industry, Tokyo, Japan). Three chlorophenoxy acid herbicides (2,4-D, Silvex and 2,4,5-T), and 4,4-dibromooctafluorobiphenyl as internal standard were purchased from Chem Service (West Chester, PA, USA). The surrogate *tert*.-octylphenoxy acetic acid (4-t-OP1EC) was synthesized as described by Fujita [16].

2.3. Sample extraction

The procedure used graphitized carbon black cartridges (GCB or ENVI-carb, trade name from Supelco, USA) to extract the compounds containing carboxylic acids from the water samples has been reported elsewhere [8,12,14]. Briefly, acidified 500-ml spiked samples were passed through the GCB cartridge at a flow-rate of about 10–20 ml/min with the aid of a vacuum. The chlorophenoxy acid herbicide residues were eluted from the cartridge with 7 ml of methylene chloride–methanol (9:1, v/v) eluent modified with 25 m*M* formic acid. The extract of the herbicide residues was then completely evaporated to dryness by a stream of nitrogen. The residues were then redissolved in 100 μl of chloroform with internal standard and 15 m*M* TBA-HSO_4, and made ready for GC–MS analysis.

2.4. GC–MS analysis

Analyses were performed on a Varian 3400CX gas chromatograph directly connected to a Saturn 2000

ion-trap mass spectrometer (Varian, USA). A large-volume DSI (ChromatoProbe, from Varian) with an injection-port derivatization procedure has been applied and described elsewhere [11,12]. A 10–20 μl sample extract was introduced into a DSI micro sample vial. The vial was placed in the probe's vial holder and pushed into the heated zone of the injection port. The temperature of the injector was held at 80°C for 2 min to evaporate the solvent, then rapidly heated to 300°C, and held for another 30 min. The split ratio of injector was 1:10. A DB-5MS capillary column (30 m×0.25 mm I.D., 0.25 μm film, from J&W, USA) connected to 2–4 m of deactivated fused-silica per-column (as retention gap), was used. After the injector temperature had reached 300°C, the GC temperature program began as follows: 80°C for 3 min, followed by a 8.5°C/min ramp to 300°C, and hold for 4 min. At the end of the analysis, the sample vial was removed from the DSI vial holder, and disposed of. The transfer line was set at 280°C. Full scan electron impact ionization (EI) MS data were acquired under the following conditions: mass range 50–550 m/z, scan time 1 s, manifold temperature 120°C, emission current 10 μA, automatic gain control (AGC) target 25 000 (represents the target total ion current value).

The herbicide residues quantitation was calculated from the four-level calibration curve (or average response factor) covering the range 5 to 100 ng/μl, each divided by the fixed concentration of internal standard. The response factors (RFs) were calculated as follows:

$$RF = [(A_x)(Q_{is})]/[(A_{is})(Q_x)]$$

where: A_x = peak area of the characteristic mass chromatograms of the herbicide, A_{is} = peak area of the characteristic mass chromatogram of internal standard, Q_x = concentrations of the herbicide standard (ng/μl) and Q_{is} = fixed concentration of internal standard (ng/μl).

The precision of the curve, as indicated by the relative standard deviation (RSD) of response factors, was 4%, 8% and 8% for butylated 2,4-D, Silvex and 2,4,5-T, respectively. The precision of the injection-port derivatization and GC–MS analysis, determined from the RSDs of over 80 injections of the herbicides, ranged from 4 to 13%.

3. Results and discussion

3.1. Evaluation of tetraalkylammonium salts and injector port conditions

Owing to the availability of different TAA salts and the possible dependence of derivatization efficiency on reagent selection, two TAA salts (TBA-HSO_4 and TMA-HSO_4) were initially evaluated regarding their reaction with phenoxy acids to form their corresponding alkyl esters. Of the two reagents tested, TBA-HSO_4 was chosen because butylated phenoxy acids produced the highest molecular ion peaks in the characteristic mass chromatogram and improved the determination sensitivity. No retention effect of TAA salts in the injection port was detected since the DSI device with disposable micro vial was used and no glass wool was inserted into the inlet glass liner. Therefore, this method did not require a routine check of sample carryover by subsequent injection of a different ion-pair reagent after a sample injection, as described by Field and co-workers [17,18]. Meanwhile, the sharp and symmetric peaks remained visible after a series of 50 sample injections.

To enhance sensitivity, the 100 μl of sample volume could be introduced by five cycles of sample loading and solvent evaporation. Between each cycle, 20 μl sample extract was renewed in the micro vial. Although the results demonstrated that introduction of up to 100 μl is possible using the DSI device, this study considers 20 μl to be the routine analysis choice because it can achieve rapid results and sufficient quantitation limits. This study also evaluated the effect of TBA concentration. Among the four concentrations (10, 15, 20 and 30 mM), 15 mM was selected because it produced the highest average peak areas of the butylated chlorophenoxy acid herbicides. Details on how to evaluate the conditions of the injection port can be found elsewhere [11,12]. This work employed an injection temperature of 300°C following the injector-temperature program as the Experimental section describes.

3.2. GC–MS of phenoxy acid herbicide residues

Fig. 1 depicts the full-scan EI mass spectra of the

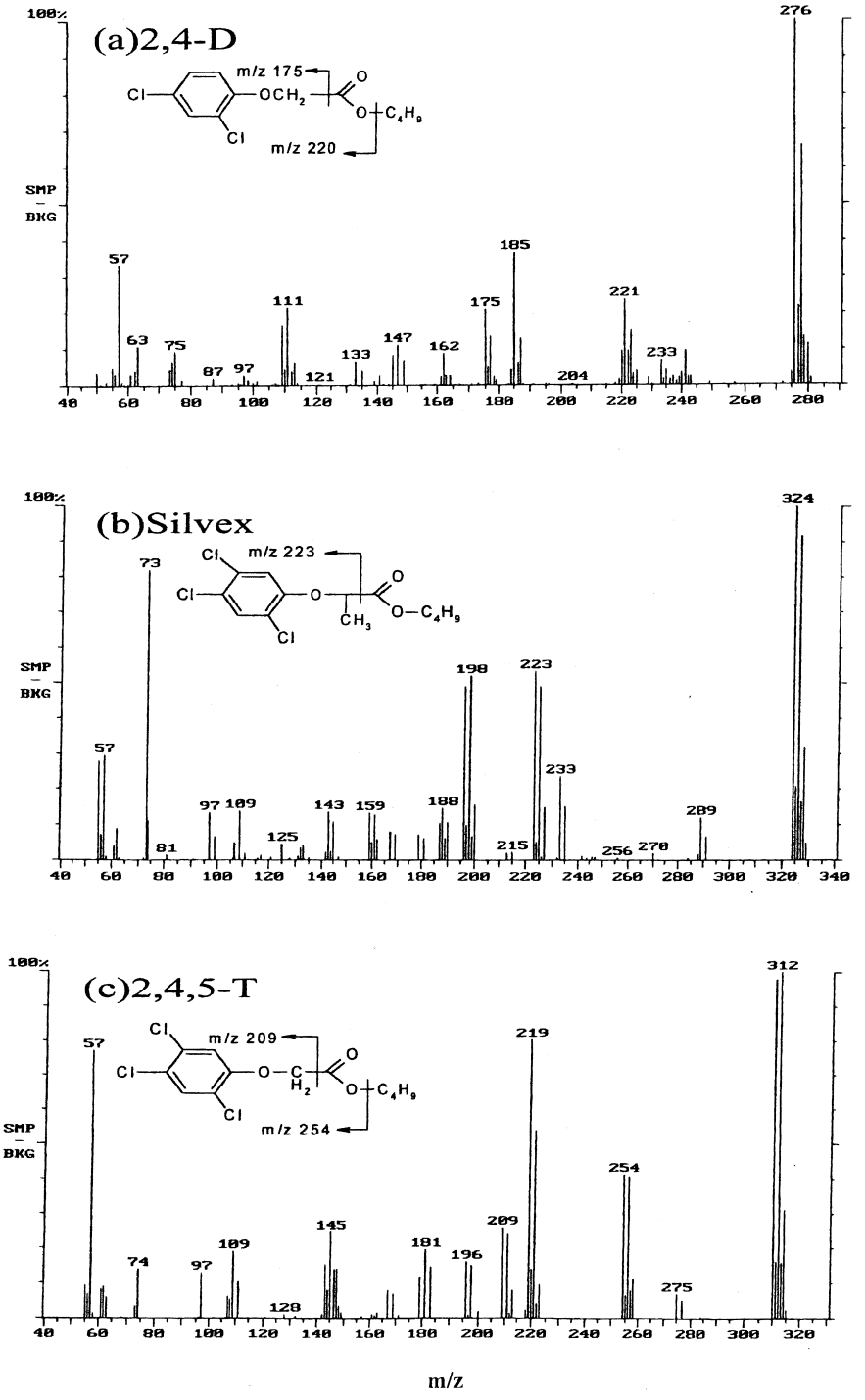

Fig. 1. Full-scan EI mass spectra of butylated (a) 2,4-D, (b) Silvex and (c) 2,4,5-T detected from a 500-ml river water spiked with 1 μg/l of the analytes.

butylated 2,4-D, Silvex and 2,4,5-T by TBA-HSO_4. Intense molecular ions with significant chlorine isotope patterns of these butylated herbicides were observed. The relative abundance of characteristic $[M-56]^+$, $[M-56-Cl]^+$ and $[M-101]^+$ ions was determined due to the loss of a butene from the butyl ester side, loss of butene plus chlorine and loss of the $HCOOC_4H_9$ group, respectively. Meanwhile, the molecular mass determination of chlorophenoxy acid herbicides in the complex environmental samples can easily be confirmed by the ions of $[M-56]^+$ and $[M]^+$. Fig. 2 displays the characteristic mass chromatograms of butylated 2,4-D, Silvex and 2,4,5-T extracted from a river water spiked with the herbicides. The individual compounds are clearly displayed by the peaks representing the intense molecular ions at m/z 276, 324 and 310, which can be used to characterize 2,4-D, Silvex and 2,4,5-T in complex environmental matrices, respectively.

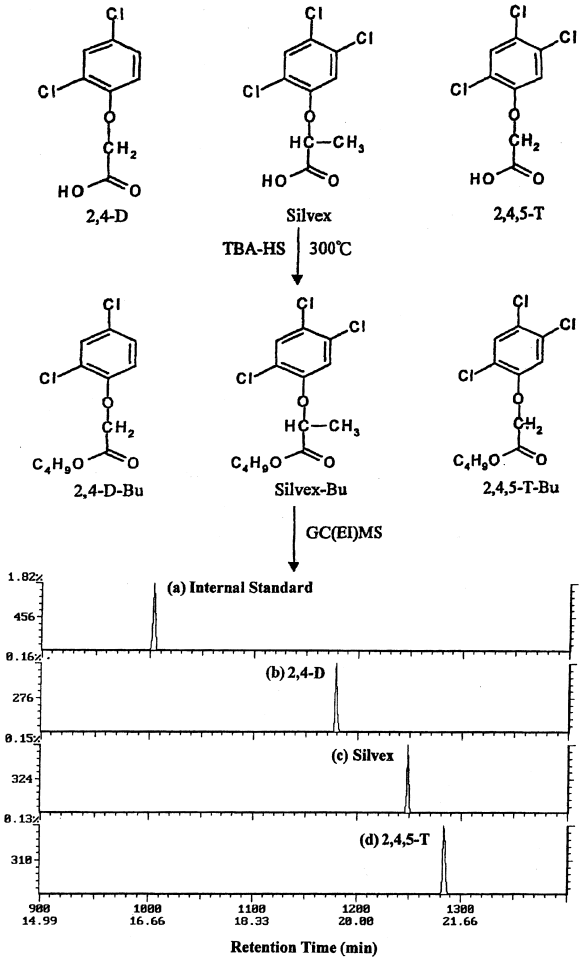

Fig. 2. Flow diagram of the on-line derivatization and the characteristic mass chromatogram of the butylated herbicides isolated from a 500-ml river water spiked with 1 μg/l of the analytes.

3.3. Recovery study and application of the method

The quantitation limit of butylated 2,4-D, Silvex and 2,4,5-T by EI-MS was 0.1 to 0.2 μg/l in 500-ml water samples, defined at a signal-to-noise ratio ($S/N \geq 10$. The recovery from GCB SPE was evaluated using deionized water spiked with a known amount of 2,4-D, Silvex, 2,4,5-T and 4-t-OP1EC (as surrogate). Seven replicate 500-ml deionized water samples were individually spiked to obtain final concentrations of 1 μg/l of 2,4-D, Silvex, 2,4,5-T and 4-t-OP1EC. Recovery of butylated 2,4-D, Silvex and 2,4,5-T in spiked deionized water samples was 95% (RSD = 6%), 92% (RSD = 6%) and 75% (RSD = 6%), respectively. The recovery of butylated surrogate 4-t-OP1EC was 99% with a 5% RSD. The recoveries proved to be satisfactory and there were no significant differences in the average peak areas when samples were acidified to pH from 1.5 to 4.0.

No chlorophenoxy acid herbicide residues were detected in the river samples. The average precision ($n = 6$) of the method applied to river water fortified with 1 μg/l of the analytes using internal calibrations and internal standards was determined to be 10% for 2,4-D, 7% for silvex, and 13% for 2,4,5-T. Table 1 summarizes the average recovery of these three herbicides in the fortified environmental samples. The recoveries are decreasing with increasing number of chlorine atoms in the molecule, which maybe due to the acid strength as described by Notel and Kruger for phenoxy acidic herbicides [19] and Butte et al. for phenols and fatty acids [20]. The results indicate that the direct injection-port derivatization procedure with the ion-pair reagent simplified the preparation of the calibration standard and the quantitation of the samples.

In conclusion, this work demonstrates that GCB SPE and injection-port derivatization using a large-volume DSI device with TBA salts, is an effective method for the rapid determination of trace levels of

Table 1
Recovery results of investigated herbicides spiked into various water samples[a]

Sample	2,4-D	Silvex	2,4,5-T	4-t-OP1EC (surrogate)
Deionized water ($n=7$)				
Spiked recovery (%)	95 (6%)	92 (6%)	75 (6%)	99 (5%)
River water-A ($n=3$)				
Background concentration (μg/l)	n.d.	n.d	n.d.	80 (11%)
Spiked recovery (%)	89 (10%)	75 (7%)	75 (13%)	83 (5%)
River water-B ($n=3$)				
Background concentration (μg/l)	n.d	n.d	n.d	89 (6%)
Spiked recovery (%)	99 (1%)	70 (4%)	75 (6%)	85 (6%)
Quantitation limit (μg/l)	0.1	0.1	0.2	

[a] The relative standard deviation (RSD) is given in parentheses, n.d.: not detected at method quantitation limit.

chlorophenoxy acid herbicides in aqueous samples. The method significantly cuts the solvent waste and simplifies sample preparation, typically avoiding derivatization with hazardous reagents in current use. Furthermore, the application of internal calibration and surrogate recovery compensate the possible analyte losses, and provide high precision, as well as quality control. The method can be used as a rapid screening tool, and to obtain detailed information on the sources, behavior and fate of the acidic herbicide residues in both surface water and groundwater.

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