Isolation and Recovery From Water of Selected Chlorophenoxy Acid Herbicides and Similar Weak Acid Herbicides by Solid-Phase Extraction HPLC and Photodiode Array Detection

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ISOLATION AND RECOVERY FROM WATER OF SELECTED CHLOROPHENOXY ACID HERBICIDES AND SIMILAR WEAK ACID HERBICIDES BY SOLID-PHASE EXTRACTION HPLC AND PHOTODIODE ARRAY DETECTION

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ABSTRACT

Solid-Phase Extraction (SPE) has been combined with High Performance Liquid Chromatography using a Photodiode Array Detector to isolate, recover and quantitate selected Chlorophenoxy acid herbicides, dicamba and dinoseb from spiked deionized water and a drinking water sample. Percent recoveries have been determined for spiked water and the precision for replicate SPEs has been determined. The capability of photodiode array detection in providing qualitative analysis and in increasing sensitivity via wavelength programming has been demonstrated. A method is proposed which eliminates non-polar matrix interferences. Recoveries for selected Chlorophenoxy acid herbicides were not influenced using this method.

INTRODUCTION

Chlorophenoxy acid herbicides (CPHs) are widely used to control weed growth and thus pose a threat to drinking and groundwater supplies.

1297

Unfortunately, the conventional method for trace CPH residue analysis is time consuming, uses toxic solvents and reagent, and in our laboratory, the method exhibited inconsistent percent recoveries on matrix spiked samples (1-3).

The conventional method requires liquid-liquid extraction (LLE), acid-base partitioning, methyl ester derivatization and cleanup. In addition, packed column gas chromatography with electron-capture detection is used (P-GC-ECD). We have recently reported on the use of dual wide-bore capillary columns (D-WB-C-GC-ECD) for trace CPH determinations (4).

High performance liquid chromatography (HPLC) via direct aqueous injection (DAI) using ultraviolet absorption (UV) is an attractive alternative to the conventional GC-ECD approaches since derivatization is eliminated. Numerous 2,4-D formulation analyses using HPLC-UV have been reported (5). Even ring hydroxylated derivatives of 2,4-D appear to be easily separated using reversed-phase high performance liquid chromatography (RP-HPLC) (6). Unfortunately, DAI combined with HPLC-UV does not reach the Method Detection Limits (MDLs) required in the conventional method nor for the more recent multi-residue CPH method of the Environmental Protection Agency, i.e. EPA Method 8150 (7).

Reversed-phase solid-phase extraction (RP-SPE) was first reported by Hoke, et al. to isolate CPHs from environmental aqueous samples using RP-SPE techniques (9). Wells, et al. have also studied 2,4-D via RP-SPE (10). Both studies used fixed wavelength UV detection. Photodiode array detection provides an opportunity for the chromatographer to explore all wavelengths in the UV and choose the monitoring wavelength which maximizes instrumental sensitivity.

Combining RP-SPE with HPLC-PDA using selective wavelength programming can overcome the sensitivity limitation of DAI-HPLC-UV discussed earlier. Loconto recently demonstrated that a MDL for 2,4-D of 1 ppb could be reached using SPE-HPLC-PDA provided at 1000:1 pre-concentration factor is attained (11). Conventional SPE manifolds are not designed to accept 1000 ml of aqueous samples. Joining a separatory funnel to a glass manifold in such as way that a column can be detached for refilling, enables 1000 ml to be passed through a RP-SPE sorbent and thus isolate CPHs from the aqueous matrix.

A valid alternative method must also provide for elimination of possible matrix interferences. CPH residues that could be present in a contaminated ground wastewater sample could be obscured. The influence of an initial LLE using methylene chloride on CPH recoveries was also studied and the results are presented.

Practical quantitation limits (PQLs) are stated for 2,4-D as 10 ppb; for 2,4,5-T as 2 ppb and for Silvex (2,4,5-Trichloro phenoxy propionic acid) as 2 ppb (8).

MATERIALS

Both pre-packed cartridges and bulk C_{18} and C_8 chemically bonded silica sorbents were used (Analytichem International, Harbour City, California). for those cartridges that we prepared, two 20 um polyethylene frits were used to hold the sorbent in place. All cartridges were conditioned with Methanol or Acidified Methanol (5% HCl in MeOH). An Adsorbex (Trademark) (E.M. Science, Cherry Hill, New Jersey) vacuum manifold was used to conduct all SPE studies reported here. HPLC separations were conducted using a Pecosphere 3 x 3 C_{18} column (Perkin-Elmer, Norwalk, Connecticut).

INSTRUMENTATION

High Performance Liquid Chromatographic System to include:

a. Bio 210 Reciprocating Pump (Perkin-Elmer, Norwalk, Connecticut)

b. Rheodyne Six-Part Injection Valve with 6 ul loop

c. LC-245 Photodiode Array Detector (Perkin-Elmer)

d. GP-100 Printer/Plotter (Perkin-Elmer)

e. NEC IV Micro-computer and CRT including Analog to Digital Interface (Data Translation) using Maxima software (Dynamic Solutions, Ventura, California)

TABLE 1

Herbicide	Elution Order	pKa*	Aqueous Solubility (mg/L)*
Dicamba	1	1.9	4500, 7900
2,4-D	2	2.8, 3.3	650, 725
2,4,5-T	3	2.8, 3.5	250, 270
Silvex	4	3.0	140
Dinoseb	5	1.5, 4.4	50

* Note: Two reference sources were used to obtain pKa and S values.

RESULTS AND DISCUSSION

Weak acid aqueous dissociation constants (pKa) and aqueous solubility(s) are two important chemical properties of CPH's and related weak acid herbicides. These properties govern the extent to which a weak organic acid can be efficiently isolated and recovered using solid-phase extraction. Table 1 presents pKa and S values for each of the five herbicides studied along with the RP-HPLC elution order.

HPLC elution order under isocratic and reversed-phase conditions for these five analytes is inversely related to their aqueous solubilities. Dinoseb, being a phenol, might be acidic enough due to the two nitro groups to possess a pKa of 1.5. Its lower aqueous solubility could be attributed to the large and hydrophobic sec-butyl substituent on the phenyl ring.

It is easily shown that the extent to which any analyte, initially dissolved in water, can be isolated on a sorbent is related to a ratio of chromatographic capacity factors provided secondary equilibrium effects are ignored in the following manner:

$$\frac{n_S}{n_A} = \frac{k^1}{1+k^1} \qquad (1)$$

where

n_S - number of millimoles of analyte A sorbed

n_A - number of millimoles of analyte A present in the volume of water passed through the SPE cartridge

k^1 - capacity factor for the cartridge

Refer to Figure 1 for a description of terms.

For example, to achieve a 90% recovery, (this assumes that the elution step removes all sorbed analyte), a sorbent must have a capacity factor of at least 9 for that particular analyte. The capacity factor in turn is related to the phase ratio and molecular distribution constant (partition coefficient) as follows:

$$k^1 = \frac{V_S}{V_M} K_D \qquad (2)$$

Table 2 provides some values for K_D and uses equation 2 to calculate the minimum phase ratio for a sorbent cartridge so as to achieve a 90% recovery.

A 40 μm, 60Å pure chemically bonded silica sorbent of mass 250 mg has V_m = 300 μL. Assuming a 20Å length for a C_{18} ligate and a 350 m^2/g surface area, this same 250 mg sorbent can be estimated to have V_S = 175 μL. We can roughly estimate a phase ratio, 175/300 = 0.6.

Referring to Table 2, an analyte's partition coefficient needs to be within the range 10-100 to realize a 90% recovery. Loconto has discussed these findings as they pertain to the observed differences in experimental percent recoveries for various priority pollutant phenolics and polycyclic aromatics (14) and for various organophosphorus pesticides (15).
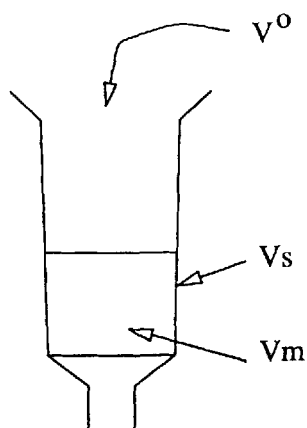
V^o - Volume of sample

V_s - Volume (reversed-pha layer) of sorbent

V_m - Void volume of sorber

n^o_A Original number of millimoles of solute A

C^o_A Original concentration of solute A in sample

n_A Number of millimoles of A sorbed into reversed-phase layer

n_m Number of millimoles of A present in void volume of sorbent

K_D Molecular partitioning or distribution constant

FIGURE 1. Schematic of a Sorbent Cartridge RP-SPE Column with Definitions used in the Text.

TABLE 2

To Achieve a 90% Recovery, i.e. $k^1 = 9$.

If K_D is	Then V_s must be
0.001	≥9000
0.01	≥900
0.1	≥90
1	≥9
10	≥0.9
100	≥0.09
1000	≥0.009

TABLE 3

SPE-HPLC-PDA.   Comparison of Elution Solvents in the Recovery of
Chlorophenoxy Herbicides

Elution Solvent	2,4-D	2,4,5-T	Silvex
Methanol	100 (3.5)	95.8 (3.3)	97.1 (5.5)
Acetonitrile	100 (3.8)	91.6 (2.8)	103 (3.8)

Notes: (1) Triplicate 6 μL injections per SPE over three C8RPSiO$_2$ (Bond
              Elute, Analytichem)
        (2) Percent RSD in parenthesis obtained by Propagating Error for
              Sample and Reference

Water

$H^+(pH < 2.0)$

Organic solvent (CPH acids & esters)

$OH^-(pH > 9.0)$

Organic phase   Aqueous phase

CPH esters (nonionic) ——————→ CPH acids as salts

Hydrolysis

H^+

Cleanup   Organic solvent (CPH acids)

GLC analysis   Derivatization (CPH esters)

Cleanup

GLC analysis

FIGURE 2. Flow Scheme for the Conventional LLE-Der-P-GC-ECD
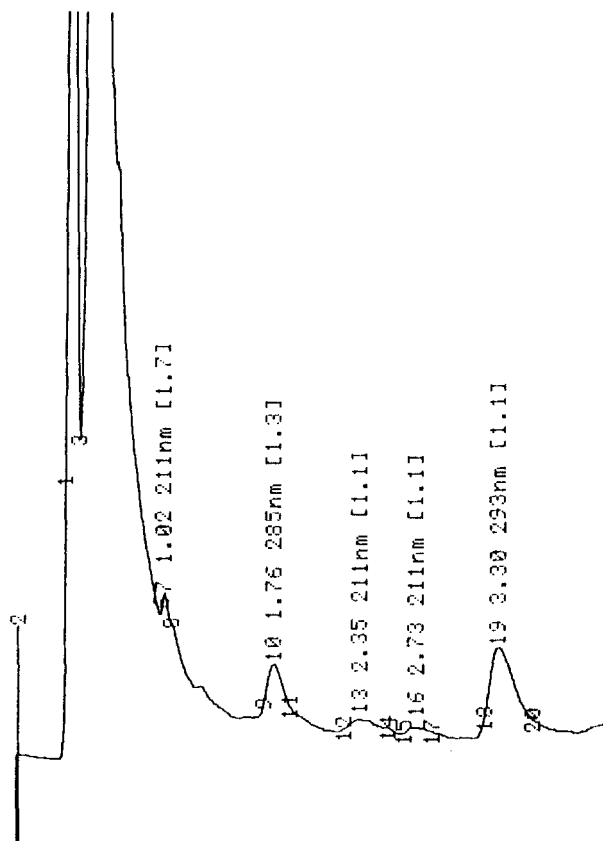Approach to CPH Trace Residue Analysis.

FIGURE 3. 500 ml of DW 0972 (Nanco Labs ID) Spiked with 350 ul of 11.6 ppm 2,4-D and 23.0 ppm Silvex (2,4,5-TP). Isolated and Recovered using C18RPSiO2.

Experience with the SPE technique in the measurement of percent recoveries for a variety of organic compounds dissolved in water leads to the conclusion that K_D values below 10 are chiefly responsible for analyte loss, hence low percent recoveries for some analytes of environmental interest. Fortunately, the three CPH's studied here are quantitatively isolated and eluted from spiked laboratory deionized water. Hence, these three CPH's have sufficiently large values for K_D.

OVERLAY SPECTRA 10: —— and 19: – – –

λ MIN: 210nm

AU

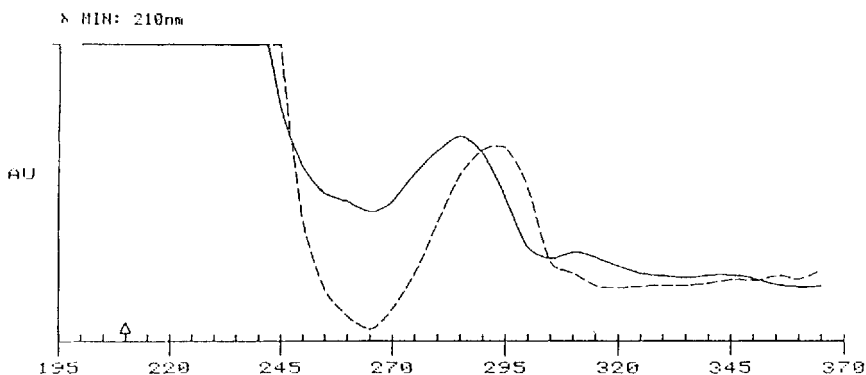195   220   245   270   295   320   345   370

FIGURE 4. Overlay of the Ultraviolet Adsorption Spectra of 2,4-D (10) and Silvex (19) Obtained from the Spiked DW0972 Sample.

Assuming a relatively large k^1 for the sorption of the three CPH's on a C_{18} sorbent, we sought to compare differences in percent recoveries between methanol and acetonitrile. Both solvents are compatible with reversed-phase HPLC. Wells and Michael (10) reported what seemed to be small differences in percent recoveries between these two elution solvents.

The consistently high percent recoveries for the three CPHs studied from spiked laboratory deionized water was found to be independent of which elution solvent, methanol or acetonitrile, was used. If a control is used to establish 100% recovery, i.e. by taking identical aliquots of spiking solution and identical final extract volumes, it is necessary to propagate the error from the two measurements, i.e. the RSD from the sample and the RSD from the control. The result of this error propagation tends to slightly increase the reported total RSD. Loconto recently took propagation of error into account when conducting recovery studies on organophosphorus pesticides via SPE (15).

The conventional method for trace CPH determinations assumes that CPH's might be present in the environment as esters or amine salts and incorporates an

TABLE 4

Effect of an Initial LLE on the Percent Recovery of CPHs.

Sample	2,4-D	2,4,5-T	Silvex
SPE-1	106 (11.3)	112 (9.9)	101 (10.7)
SPE-2	109 (5.4)	128 (4.2)	110 (6.8)

Notes: (1) 400 µL of 22.4 ppm 2,4-D, 16.8 ppm 2,4,5-T and 18.5 ppm Silvex added to DI.
(2) RSD in parentheses is for six replicate injections of sample.

alkaline hydrolysis step followed by a second LLE to recover the hydrolyzed esters as their weak acids. A schematic for this multi-step approach is shown in Figure 2. A typical chromatogram for the isolation and recovery of 2,4-D and Silvex from a drinking water sample that was spiked with these two CPH's is shown in Figure 3. The values for the peak purity index are close to one for both recovered analytes. Ultraviolet absorption spectra for 2,4-D and Silvex are overlayed as shown in Figure 4. Absorption maxima differ somewhat for these two chlorophenoxy acids as well as overall spectrum profile.

Given the excellent recoveries for the three CPH's studied from spiked DI water we sought to develop a method which would be similar in scope to the conventional LLE method discussed earlier. An important step in the new SPE-HPLC-PDA approach would be to initially hydrolyze the sample prior to SPE. We then investigated the extent of analyte loss upon an initial LLE of an alkaline spiked sample containing the three CPH's. Table 4 shows the results of this study.

These results point the way to a method whereby non-polar matrix interferences which would otherwise obscure the HPLC chromatogram are removed from the aqueous sample via LLE without CPH analyte loss.

FIGURE 5. HPLC Chromatogram: Dicamba, 2,4-D, 2,4,5-T, Silvex (4 ppm) and Dinoseb (2 ppm) in ACN 60% (1% H3PO4). 40% ACN. 2.0 mL/min, 3 x 3 Pecosphere C18.

Despite the precision being lower for replicate injection, we clearly demonstrated that the completely ionized CPH's under alkaline conditions could be quantitatively recovered.

Figure 5 is a typical HPLC chromatogram for the separation of Dicamba, the three CPH's and Dinoseb under isocratic conditions. Dicamba is a widely
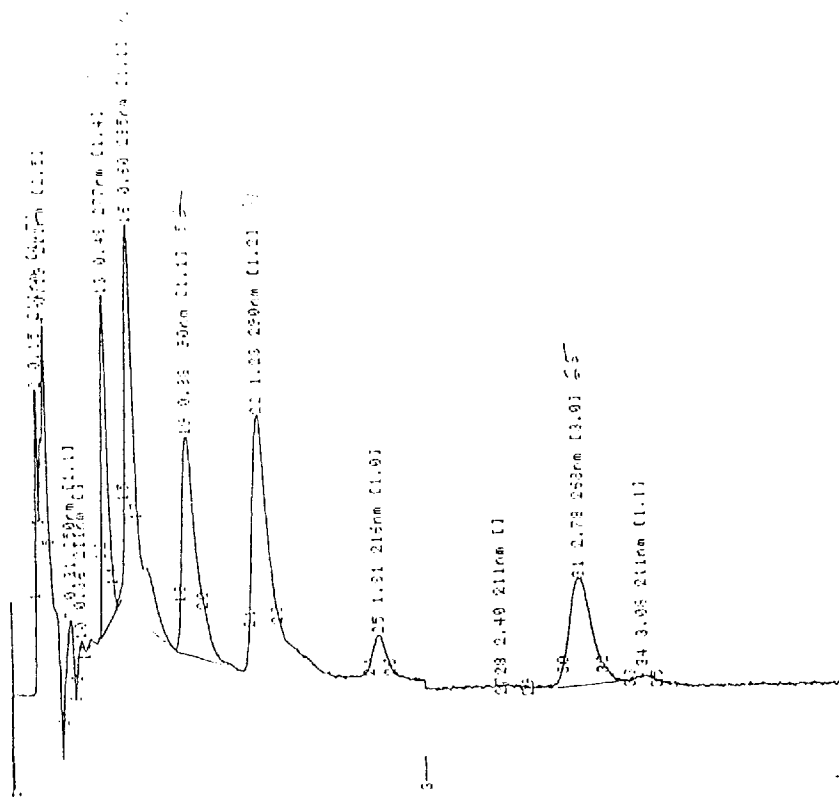
FIGURE 6. HPLC Chromatogram of Dicamba, CPH's and Dinoseb, Isolated and Recovered from DI Water using SPE.

used non-phenoxy acid herbicide while Dinoseb is a dinitro-sec-butyl substituted phenol with herbicidal properties. Dinoseb is also among the EPA's Appendix IX groundwater monitoring list (16). Figure 6 shows the five herbicides having been isolated from spiked DI and recovered using methanol. The use of a 3 μm cartridge type column resulted in a total chromatographic run time of less than three minutes. The same methanolic extract was then reinjected, but this time
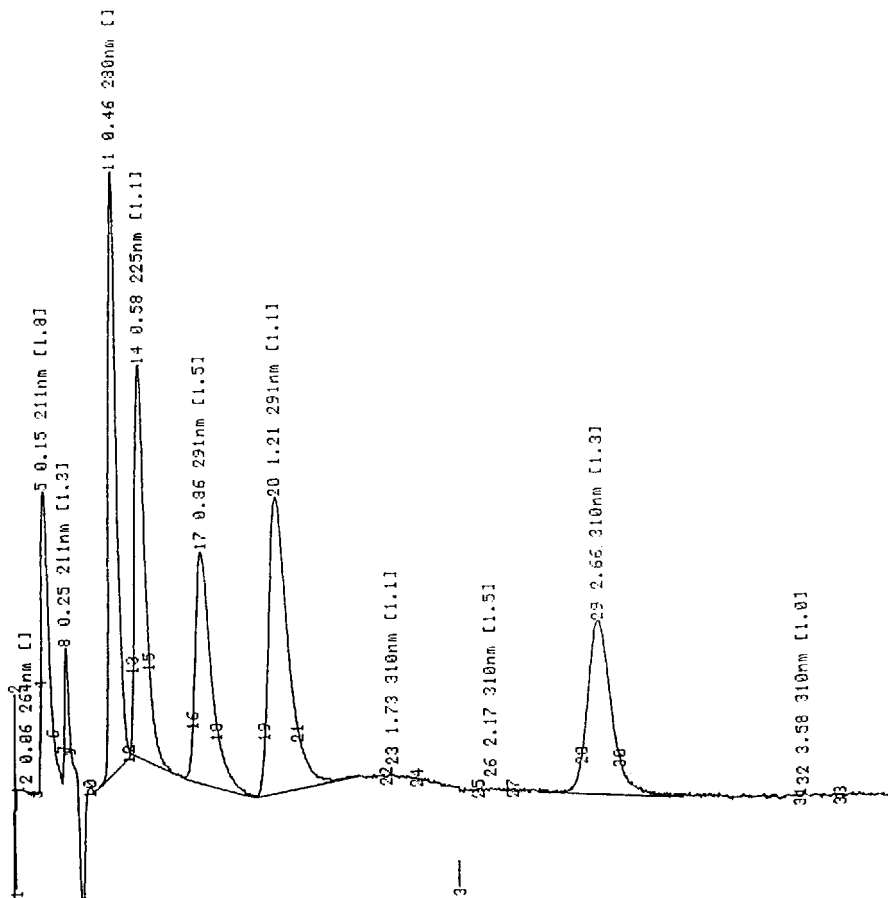
FIGURE 7. HPLC Chromatogram: Dicamba, 2,4-D, 2,4,5-T, Silvex (4 ppm) and Dinoseb (2 ppm) in ACN 60% (1% H3PO4). 40% ACN. 2.0 mL/min, 3 x 3 Pecosphere C18. Wavelength Programmed.

TABLE 5

Percent Recoveries for the Isolation of Dicamba, CPH's and Dinoseb via SPE-HPLC-PDA.

Analyte	Mean % Recovery	RSD (%)*
Dicamba	62	9.1
2,4-D	101	4.0
2,4,5-T	95.5	7.1
Silvex	91.4	3.4
Dinoseb	65.0	3.4

* Note: RSD's reflect replicate SPE's performed and do not propagate error between sample and control.

wavelength programmed and is shown in Figure 7. Note the increased signal response for all five analytes.

Using this five component mix, a preliminary recovery study was initiated and results are shown in Table 5.

The 65% recovery for Dinoseb obtained for this experiment appears to be inconsistent with the generalization about aqueous solubility and percent recovery. A more recent attempt to measure percent recoveries for Dinoseb resulted in nearly 100% values (17). This would then be consistent with the relatively low (50 ppm) aqueous solubility for Dinoseb (Table 1) and implies a higher distribution coefficient when compared to CPH's.

CONCLUSION

The following conclusions can be drawn from this work:

1. RP-HPLC using an isocratic mobile-phase and a fast C18 cartridge-type column give good resolution for at least five weak acid herbicides.

2. RP-HPLC is the most suitable instrumental method to use to study the isolation and recovery of weak acid herbicides since unlike GC methods, no derivatization/LLE is required.

3. Chlorophenoxy acids and dinoseb are quantitatively isolated and recovered from an aqueous matrix using RP-SPE techniques while dicamba, being much more soluble, is recovered to a lesser extent.

4. If a 1000:1 pre-concentration factor can be realized, MDLs for SPE-HPLC are equivalent to the conventional LLE-Der-Cleanup-GC-ECD approach with a significant savings in time, cost and solvent consumption. Toxic solvents are likewise eliminated.

5. An initial LLE to remove non-polar interferences from spiked deionized water whose pH was adjusted to 11 showed no recovery losses for the CPHs studied. Hence, the method becomes applicable to wastewater samples.

ACKNOWLEDGMENTS

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