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# Chlorinated acid herbicides in water by strong anion-exchange disk extraction and in-vial elution and derivatization

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## Abstract

An analytical method for rapidly and quantitatively detecting chlorophenoxy acid herbicides in water is presented. Strong anion-exchange, 25-mm diameter solid-phase extraction disks are used to concentrate the chlorinated acid herbicides, including Dicamba, 2,4-D, Silvex, 2,4,5-T, and Picloram, from surface water. The chlorinated acids are simultaneously eluted from the disk and derivatized to their methyl esters with an in-vial elution technique that requires heating the disk at 80°C for 1 h in the presence of 1 ml acetonitrile and 200  $\mu$ l methyl iodide. Quantitative recovery (>95%) of all the acids was achieved from 500 ml samples with a single 25 mm diameter disk. The detection limits for 2,4-D and Dicamba are 0.36  $\mu$ g/l and 0.32  $\mu$ g/l, respectively, and 0.05  $\mu$ g/l for Silvex, 2,4,5-T, and Picloram. The quantitation limit of the method is 0.50  $\mu$ g/l for Dicamba, 0.70  $\mu$ g/l for 2,4-D, and 0.13  $\mu$ g/l for Silvex, 2,4,5-T, and Picloram. With this method a total of 10 ml of solvent is required to process a 500-ml sample, sample production capacity is 20 samples/day, and diazomethane is replaced by methyl iodide. The precision of the method, indicated by the relative standard deviation, for each herbicide ranged from 0.7 to 1.0% for concentrations between 3.28 and 7.03  $\mu$ g/l and 6 to 10% for concentrations from 0.10 to 0.49  $\mu$ g/l.

**Keywords:** Water analysis; Environmental analysis; Sample preparation; Pesticides; Phenoxyacid herbicides; Chlorinated phenoxy acids

## 1. Introduction

Regulations for the monitoring of the chlorinated acid herbicides, 2,4-D and Silvex, in drinking water were established in 1991 [1]. In order to meet monitoring requirements and replace older, less efficient methodologies, new, rapid and reliable analytical methods are needed for the determination of chlorinated acid herbicides in water. The conven-

tional methods for the determination of chlorophenoxy acid herbicides in drinking water [US Environmental Protection Agency (EPA) Method 515.1] and wastewater (EPA Method 8151A) are based on liquid–liquid extraction, which require large volumes of solvents and multiple handling steps [2,3]. Alternative extraction methods have been published for isolating chlorinated acid herbicides from water but are based largely on the use of hydrophobic disks such as C<sub>18</sub> or styrene–divinylbenzene [4,5]. The use of hydrophobic disks requires the acidification of water samples prior to extraction. In addition, the conventional method for

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disk elution consists of passing solvents dropwise through the disk so that analyte elution and derivatization are separate steps. Lastly, derivatization of chlorinated acid herbicides is typically performed using diazomethane, which is a toxic and explosive reagent and the precursor reagents are carcinogenic.

Strong anion-exchange (SAX) disks are an attractive alternative for extracting chlorinated acid herbicides from water without the addition of salt or acidification. Unfortunately, analyte elution from SAX disks is reported as cumbersome and time-consuming [5,6]. However, Chatfield et al. [7] demonstrated the use of a SAX phase as a means for isolating chlorinated acid herbicides (2,4-D and 2,4,5-T) from water and as a subsequent catalyst in the reaction between exchanged chlorinated acids and the alkylation reagent methyl iodide. In a modification of the Chatfield et al. [7] approach, Field and Monohan demonstrated an "in-vial" elution technique whereby the mono- and dicarboxylated metabolites of the herbicide Dacthal (DCPA) were simultaneously eluted from SAX disks and derivatized to their ethyl esters in a single step in a gas chromatography (GC) autosampler vial [8]. The in-vial disk elution and derivatization approach is also applicable to industrial wastewaters and municipal sewage effluents. Field and Reed [9] quantitatively determined alkylphenol polyethoxy carboxylates, the metabolites of nonionic surfactants, in pulp and paper mill and municipal sewage effluents using in-vial elution and derivatization. In a pharmaceutical application, Wu et al. [10] reported a similar strategy using hydrophobic  $C_{18}$  disks to simultaneously combine the elution and derivatization of the primary metabolite of marijuana to its trimethylsilane derivative.

In this study, the SAX isolation and in-vial elution technique is extended to the determination of chlorinated acid herbicides in surface water. The chlorinated acid herbicides (Fig. 1) evaluated for this study include 2,4-dichlorophenoxy acetic acid (2,4-D), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), 3,6-dichloro-2-methoxybenzoic acid (Dicamba), 2-(2,4,5-trichlorophenoxy) propionic acid (Silvex), and 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (Picloram). The ester forms of the chlorinated acid herbicides are not considered in this study.

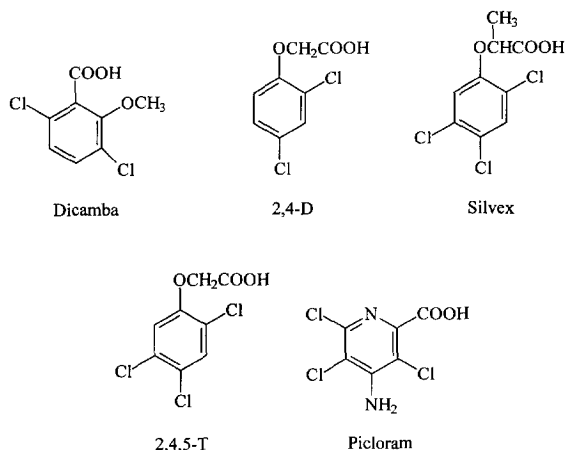


Fig. 1. Chlorinated acid herbicides investigated in this study.

## 2. Experimental

### 2.1. Standards and reagents

Standards of 2,4-D (99.9% purity), 2,4,5-T (98.7% purity), Silvex (99.3% purity), and Dicamba (97.5%) were obtained from the EPA (Research Triangle Park, NC, USA) and a standard of Picloram (99.8% purity) was obtained from Ultra Scientific (North Kingstown, RI, USA). All acid standards were prepared in acetonitrile at 30–80  $\mu\text{g/ml}$ . The dipropyl ester of the dicarboxylic acid metabolite of Dacthal was used as an internal standard in this study and was prepared using diazopropane. The acetonitrile, acetone, methanol, and toluene solvents used in this study were Burdick and Jackson GC grade (Baxter, Muskegon, MI, USA).

### 2.2. Solid-phase extraction

SAX (25 mm diameter) disks were obtained from Varian (Sugarland, TX, USA). The SAX disks are manufactured with chloride as the counter ion and were used as received. The 25-mm disks were placed in 25 mm screw-together polypropylene filter assemblies (Micro Filtration Systems, Dublin, CA, USA) and attached to a vacuum manifold (Supelco, Bellefonte, PA, USA). A 75-ml polypropylene reservoir was attached to the disk-filter assembly (Fig. 2).

Acetone (4 ml) is first applied to the disk and

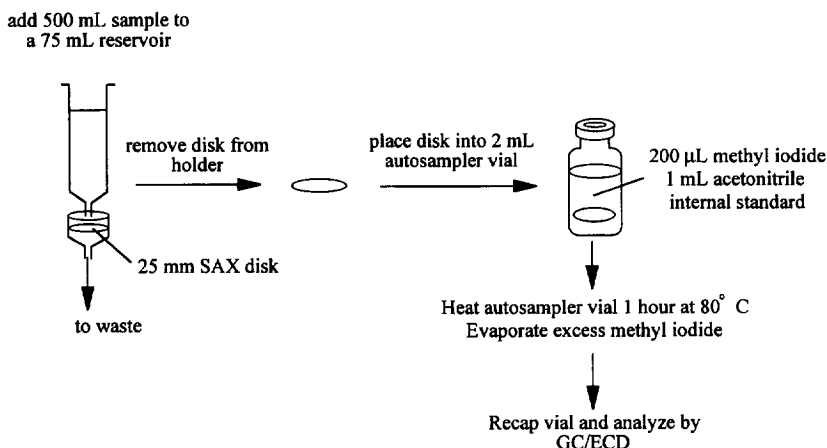


Fig. 2. Sample concentration and in-vial disk elution scheme for the analysis of chlorinated acid herbicides in water.

allowed to soak for 30 s before pulling it through the disk under vacuum. Methanol (4 ml) is then applied to the disk, allowed to soak for 30 s and then drawn through the disk leaving a small amount to cover the disk without allowing the disk to dry. Two 5-ml rinses of deionized water are pulled through the disk. The sample is then added to the reservoir and pulled through the disk under full vacuum ( $-10^5$  Pa). The sample bottle is rinsed with deionized water ( $2 \times 15$  ml) and added to the reservoir. Once the sample has passed through the disk and the reservoir has been rinsed with  $2 \times 5$  ml deionized water, the reservoir is removed and the process of disk drying is initiated by pushing 10 ml of air through the disk using a small hand-held syringe. The disk is then dried under full vacuum for 15 min.

To perform in-vial derivatization and elution, the dry disk is removed from the extraction assembly, folded in half, and rolled so that it can be placed in a 2-ml autosampler vial. Next, 1 ml acetonitrile, 200 µl methyl iodide and the internal standard are added to the vial, which is then capped and heated at 80°C for 1 h. Once the vial cools, the cap is removed and the vial contents are evaporated to near dryness under a stream of nitrogen in order to remove the excess methyl iodide. Leaving the disk in the autosampler vial, approximately 1 ml of toluene is added back into the vial and the vial is recapped and placed directly on the autosampler for analysis. The procedure is quite simple, as disk elution, analyte

derivatization, and solvent exchange are performed in a single autosampler vial.

Blank extractions were first performed to determine if a local surface water (Oak Creek) contained any of the chlorinated acid herbicides. The Oak Creek sample contained particulate matter but was not filtered prior to extraction. Duplicate 500-ml samples were extracted and found to contain no chlorinated acid herbicides above detection. Therefore, Oak Creek surface water was subsequently used as a blank to determine the accuracy, precision, and the detection and quantitation limits of the method.

To determine if breakthrough occurs, a single 500-ml sample of Oak Creek surface water was spiked to give concentrations of 5 µg/l of 2,4,5-T, Silvex, and Picloram and 10 µg/l of 2,4-D and Dicamba. The sample was passed through two 25-mm SAX disks stacked together in the filter assembly. Once the water had passed through the stack, the disks were separated and eluted separately by in-vial elution.

To demonstrate the accuracy and precision of the method, two sets of replicate extraction experiments were performed at low and high concentrations of each chlorinated acid herbicide. At the higher concentration, five replicate samples of Oak Creek surface water were spiked with Dicamba (4.65 µg/l), 2,4-D (7.03 µg/l), Silvex (4.53 µg/l), 2,4,5-T (5.24 µg/l) and Picloram (3.28 µg/l). Five replicate samples of Oak Creek surface water were spiked to give lower concentrations of Dicamba (0.93 µg/l),

2,4-D (1.41  $\mu\text{g}/\text{l}$ ), Silvex (0.45  $\mu\text{g}/\text{l}$ ), 2,4,5-T (0.52  $\mu\text{g}/\text{l}$ ) and Picloram (0.49  $\mu\text{g}/\text{l}$ ). The average recovery, standard deviation and relative standard deviation were calculated from the 5 replicate sample extractions and analyses.

To determine the detection and quantitation limits of the method, 500-ml samples of Oak Creek surface water were spiked with 0.001 to 0.4 mg total mass of each acid herbicide standard to give water sample concentrations from 0.002  $\mu\text{g}/\text{l}$  to 0.8  $\mu\text{g}/\text{l}$ . The samples were analyzed and the signal-to-noise ratio ( $S/N$ ) was determined for acid herbicide at each concentration. The detection and quantitation limits of the method were then defined as that concentration of acid herbicide necessary to produce a  $S/N$  of 3 and 10, respectively.

In order to calculate the method detection limit (MDL) according to the procedure given in Glaser et al. [11], seven replicate extractions were performed at concentrations that corresponded to a  $S/N$  of 5. The concentrations used for calculating the MDL included 0.37  $\mu\text{g}/\text{l}$  Dicamba, 0.49  $\mu\text{g}/\text{l}$  2,4-D, and 0.1  $\mu\text{g}/\text{l}$  for Silvex, 2,4,5-T, and Picloram. A separate five-point calibration curve was constructed to quantitate the low concentrations of the chlorinated acid herbicides used for determining the MDL. A total of 0.0007 to 0.05  $\mu\text{g}$  of each chlorinated acid herbicide was combined with 0.06  $\mu\text{g}$  of the internal standard to construct low-level quantitation standards.

### 2.3. Gas chromatography (GC)

All extracts were analyzed using a Hewlett-Packard Model 5890 Series II gas chromatograph equipped with an HP-5 column (30 m $\times$ 0.25 mm, 0.25  $\mu\text{m}$  film thickness; Hewlett-Packard, Wilmington, DE, USA). The initial oven temperature of 120°C was held 1 min and then increased at 15°C/min to 180, followed by a second ramp of 5°C/min to 250°C, followed by a third and final ramp of 30°C/min to a final temperature of 300°C. An injection volume of 1  $\mu\text{l}$  was used under splitless conditions with an injector temperature of 280°C. The  $^{63}\text{Ni}$  electron-capture detection (ECD) system was operated at a temperature of 300°C with nitrogen as makeup gas.

### 2.4. Quantitation

Diazomethane was used to prepare the standards for the quantitation curve since derivatization with diazomethane is the conventional procedure for the derivatization of chlorinated acid herbicides. Calibration curves were constructed from quantitation standards containing from 0.2–3.0  $\mu\text{g}$  of each of the chlorinated acid herbicides and spiked with 1.2  $\mu\text{g}$  of the internal standard. Conventional internal standard quantitation was used and gave linear calibration curves, typically with  $r^2$  of 0.9999. To determine the recovery of the acid herbicides, the peak areas of each chlorinated acid herbicide were divided by that of the internal standard and the corresponding concentrations were calculated from calibration curves.

## 3. Results and discussion

A single 500-ml sample of Oak Creek surface water, previously determined to be blank, was fortified with all five chlorinated acid herbicides and processed through two stacked 25-mm SAX disks to determine if breakthrough occurred. Greater than 90% of each chlorinated acid herbicide was recovered from the top SAX disk while none was detected on the bottom SAX disk indicating that breakthrough did not occur. A sample volume of 500 ml was subsequently used for all spike and recovery experiments.

The conditions reported in Field and Monohan [8] including a reaction temperature of 80°C for a period of 1 h gave quantitative (95%) recovery of all the chlorinated acid herbicides tested in this study. Initial experiments indicated that regardless of whether the internal standard was added before or after the derivatization reaction, the recovery of the acid herbicides was essentially equivalent. This finding suggests that the chlorinated acid herbicides are quantitatively eluted from the disk during in-vial elution and derivatization. Because the SAX-catalyzed reaction with methyl iodide is not a transesterification reaction, the internal standard can be added prior to reaction. In this study, the internal standard was added prior to reaction for convenience.

The accuracy and precision of the method was

Table 1  
Recovery of five chlorinated acid herbicides from 500-ml samples of Oak Creek surface water spiked at two concentrations<sup>a</sup>

Chlorinated acid	Concentration ( $\mu\text{g/l}$ )	Recovery (%)	Concentration ( $\mu\text{g/l}$ )	Recovery (%)
Dicamba	4.65	95.0 $\pm$ 0.9 (1.0%)	0.93	99.5 $\pm$ 4.3 (4.3%)
2,4-D	7.03	101.9 $\pm$ 1.0 (1.0%)	1.41	101.1 $\pm$ 1.3 (1.3%)
Silvex	4.53	98.1 $\pm$ 1.0 (1.1%)	0.45	107.0 $\pm$ 4.0 (3.8%)
2,4,5-T	5.24	99.2 $\pm$ 0.9 (1.0%)	0.52	106.1 $\pm$ 10.2 (9.6%)
Picloram	3.28	97.9 $\pm$ 0.7 (0.7%)	0.49	102.5 $\pm$ 3.5 (3.4%)

<sup>a</sup> Five replicate extractions were performed at the two concentrations listed for each chlorinated acid. The relative standard deviation (R.S.D.) is given in parentheses.

tested by performing five replicate extractions at each of two chlorinated acid herbicide concentrations (Table 1). The recovery of the chlorinated acid herbicides, spiked into water to give concentrations from 3.28–7.03  $\mu\text{g/l}$ , ranged from 95 to 102%. The precision of the method, indicated by the relative standard deviation, was 0.7 to 1.1%. A typical GC–ECD chromatogram for samples spiked at these chlorinated acid herbicide concentrations is shown in Fig. 3. At lower concentrations the recoveries ranged from 99 to 107% for samples containing 0.49 to 1.4  $\mu\text{g/l}$  of the chlorinated acid herbicides (Table 1). The relative standard deviation of replicate analyses at the lower acid herbicide concentrations ranged from 1.3 to 9.6%. Quantitative and reproducible

recovery was obtained despite the fact that the Oak Creek surface water contained particulate matter, indicating that the particulate matter did not interfere with either extraction or reaction efficiency.

The effect of pH and ionic strength on chlorinated acid herbicide recovery was not investigated in this study. However, Field and Reed [9] obtained high recoveries (>90%) for alkylphenol polyethoxy carboxylates which are also monoprotic, weak acids using SAX disks with environmental samples ranging in pH from 6 to 8.3 and with specific conductances from 140 to 380  $\mu\text{S/cm}$ .

The detection limits of the method, defined at a *S/N* of 3, are 0.32  $\mu\text{g/l}$  for Dicamba, 0.36  $\mu\text{g/l}$  for 2,4-D, and 0.05  $\mu\text{g/l}$  for Silvex, 2,4,5-T and Picloram. The quantitation limits of the method, defined at a *S/N* of 10, are 0.50  $\mu\text{g/l}$  and 0.70  $\mu\text{g/l}$  for Dicamba and 2,4-D, respectively, and 0.13  $\mu\text{g/l}$  for Silvex, 2,4,5-T, and Picloram.

To calculate the MDL, the average recovery and standard deviation were determined for the 7 replicate extractions performed at chlorinated acid herbicide concentrations that corresponded to a *S/N* of 5. The fortification level was corrected for recovery and the calculated standard deviation of the corrected average recovery was multiplied by a factor of 3.14 (the Student's *t* value for a one-tailed test at the 98% confidence interval with 6 degrees of freedom) to obtain the MDL for each acid herbicide [11]. The MDLs for the individual chlorinated acid herbicides ranged from 0.02  $\mu\text{g/l}$  for Silvex to 0.10  $\mu\text{g/l}$  for 2,4-D (Table 2) and are lower than those reported by Hodgeson et al. [5] who used a 47-mm  $\text{C}_{18}$  disk and separate steps for elution and derivatization. In addition, the MDLs determined for the chlorinated acids selected for this study are lower than the MDLs

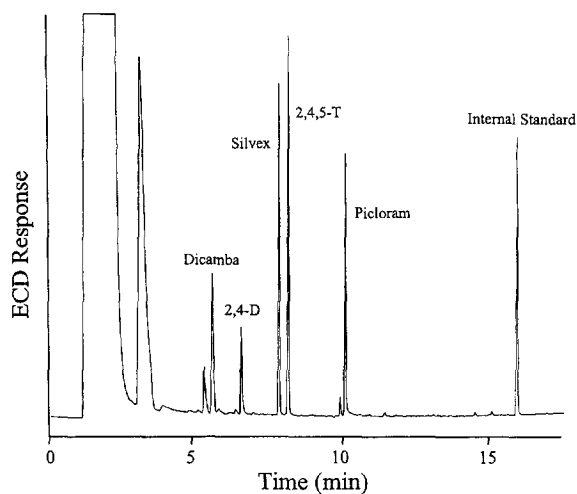


Fig. 3. A typical GC–ECD chromatogram of Oak Creek surface water spiked to give concentrations of 4.7  $\mu\text{g/l}$  Dicamba, 7.0  $\mu\text{g/l}$  2,4-D, 4.5  $\mu\text{g/l}$  Silvex, 5.2  $\mu\text{g/l}$  2,4,5-T, and 3.3  $\mu\text{g/l}$  Picloram.

Table 2

Recovery, standard deviation, and method detection limit (MDL) determined for each chlorinated acid herbicide spiked into blank Oak Creek surface water<sup>a</sup>

Chlorinated acid herbicide	Spike concentration ( $\mu\text{g/l}$ ) <sup>b</sup>	Mean recovery (%)	R.S.D. (%)	MDL ( $\mu\text{g/l}$ )
Dicamba	0.37	95.4	6.6	0.07
2,4-D	0.49	96.3	7.1	0.10
Silvex	0.10	88.3	6.8	0.02
2,4,5-T	0.10	109.9	6.8	0.03
Picloram	0.10	93.0	10.2	0.03

<sup>a</sup> Seven replicate samples were analyzed.

<sup>b</sup> Each acid herbicide was spiked at a concentration that gave a S/N of 5.

for EPA Method 515.1 [2] for drinking water and EPA Method 8151A [3] for wastewater.

#### 4. Conclusions

Five chlorinated acid herbicides were quantitatively extracted from 500-ml water samples using a 25-mm SAX disk followed by in-vial elution. The entire method consumes a total of 10-ml solvent to process a 500-ml sample and the need for diazomethane is eliminated by the use of methyl iodide. Because sample throughput is 20 samples/day, the method is more rapid than EPA Method 515.1 or 8151A, which can be used to process approximately 8 samples/day. The method is simpler to perform than EPA Method 515.2 which uses a 47-mm C<sub>18</sub> disk because analyte elution from the SAX disk is coupled with derivatization, thus saving time and reducing the number of preparative steps. Additional work is required to modify the method to include the ester forms of the chlorinated acid herbicides.

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