Solid-Phase Extraction of Dicamba and Picloram from Water and Soil Samples for HPLC Analysis†

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Methods are presented for the analysis of two commonly used herbicides, dicamba and picloram, in water and soil samples. The methods utilize solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC). For separation and concentration of the herbicides, two types of SPE cartridges were used—aminopropyl (NH_2) weak anion exchange adsorbent for dicamba and octadecyl (C_{18}) strong polar adsorbent for picloram. Detection limits for HPLC analysis of dicamba were 1 ppb for water and 10 ppb for soil samples. Recovery experiments for dicamba indicated 90–99% recovery for the concentration range 10–60 ppb in water samples and 83 ± 6% recovery of 10 ppb of dicamba added to soil samples. Detection limits for picloram were 8 ppb for water and 10 ppb for soil samples. Recovery of picloram from water samples was between 85 and 96% for the 10–60 ppb concentration range and 88 ± 6% recovery of 10 ppb of picloram added to soil samples. The recovery of standard solutions by different brand name SPE cartridges was also tested. Differences in the efficiencies of various SPE cartridges were determined, not only among manufacturers but also between lots. It is suggested that one brand name of SPE cartridge, all of the same lot number, be used throughout a particular study and that no changes in manufacturers and lots be made without adequate evaluation of the SPE cartridges for their ability to separate and concentrate the pesticide of interest.

Keywords: *Dicamba; picloram; SPE cartridges; HPLC; soils; water samples; recovery studies*

INTRODUCTION

Herbicide residue analysis generally requires several steps, such as extraction of the pesticide from the sample of interest, removal of interfering coextractives, and identification and quantification of the pesticide content (Das, 1981). There are many methods of analyzing herbicides in environmental samples, the most common being gas chromatography and high-performance liquid chromatography (HPLC). Both liquid–liquid and solid-phase extraction of solution sample can be used to extract, concentrate, and purify herbicides (Majors, 1992). The advantages of solid-phase extraction (SPE) over liquid–liquid extraction include decreased use of and exposure to hazardous materials, shorter time requirements, and no hindrance of the extraction by the formation of emulsions (Johnson et al., 1991).

With the SPE chromatography method, pesticides in aqueous samples can be isolated, concentrated, and purified. The following steps are generally required: sample preparation (mainly by pH adjustment); cartridge preparation (activation with strong solvent, e.g., methanol or acetonitrile, followed by a rinse step that removes the activation solvent); sample application (controlled by flow rate); analyte elution (by strong solvent, e.g., 25% acetic acid or methanol); analyte concentration (mainly through evaporation). Solid-phase extraction using octadecyl (C_{18})-bonded porous silica columns has been used for herbicide extraction and cleanup (Junk and Richard, 1988; Huang and Pignattelo, 1990). Herbicide extraction by SPE has also been reported for picloram in water and soil (Wells, 1986; Wells and Michael, 1987; Michael et al., 1989) and for dicamba in water (Arjmand et al., 1988).

The objectives of this study were to evaluate and improve upon methods used for the extraction of dicamba and picloram in water samples by using SPE followed by HPLC analysis. In addition, methods are presented for the extraction and analysis of dicamba and picloram from soils.

MATERIALS AND METHODS

Reagents and Equipment. Organic solvents used in the SPE and HPLC studies were of reagent grade quality suitable for trace pesticide analysis, which were obtained from Chem Service Inc. (West Chester, PA). Stock solutions were prepared by dissolving 0.1031 g of dicamba (2-methoxy-3,6-dichlorobenzoic acid) in methanol (1000 ppm) or by dissolving 0.0105 g of picloram (4-amino-3,5,6-trichloropicolinic acid) in 25% glacial acetic acid (100 ppm). Appropriate amounts of each of these stock solutions were added to water to obtain desired final concentrations. Working standards were prepared weekly for dicamba and every 2 weeks for picloram. The SPE cartridges used in the final analysis of dicamba and picloram in this study were aminopropyl (500 mg) cartridges obtained from J. T. Baker Inc. and octadecyl (1000 mg) cartridges purchased from Burdick and Jackson Corp. Solvent reservoirs, adapters, and a vacuum manifold with 12 ports were also purchased from Burdick and Jackson. Pesticide quantification was performed on a high-performance liquid chromatography (HPLC) system (Beckman Model 344 CRT-based gradient, with a 5-μm octadecyl column (25 × 0.46 cm) and a reversed-phase guard column).

Sample Preparation. Water samples to be analyzed for dicamba did not require any pretreatment. However, water samples containing picloram required the addition of 2.5 g of NaCl to 50-mL samples followed by acidification to pH 2. Extraction of dicamba from soil was based on the method of K. Luong (personal communication), whereas extraction of

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Table 1. Extraction of Dicamba and Picloram from Soil Samples

extraction	dicamba (adapted from K. Luong, personal communication)	picloram [adapted from Cheng (1969)]
amount of soil (g)	25	100
extraction solution	80 mL of methanol/water (50:50 v/v)	100 mL of 2 N KCl
process of extraction	ultrasonic vibration for 40 min; centrifuge at 2000 rpm for 20 min and at 10 000 rpm for 30 min; decant 20-mL aliquot and dilute to 100 mL with distilled water	pH adjusted to 7 with 5 N KOH; shaken for 60 min; centrifuge at 1500 rpm for 10 min and at 10 000 rpm for 15 min; decant 50 mL

Table 2. Solid-Phase Extraction Conditions for Water Samples

extraction conditions	dicamba	picloram
cartridge	500-mg aminopropyl NH_2 column	1000-mg octadecyl C_{18} column
column preparation	elute with 18 mL of 1 N acetic acid at a rate of 5 mL/min; pass 6 mL of distilled water at rate of 5 mL/min; keep 1 mL of solution above the packing material at all times	elute with 10 mL of methanol; elute 10 mL of 4% acetic acid at a rate of 12 mL/min; keep 1 mL of solution above the packing material at all times
sample application	50 mL suction-filtered at 2–3 mL/min	50 mL suction-filtered at 5 mL/min
column wash	rinse with 3 mL of methanol; dry for 15 min using the vacuum source	dry for 10 min using the vacuum source
analyte solution	elute with 2 mL of 0.1 N K_2HPO_4	elute with 4 mL of 25% acetic acid in water

Table 3. High-Performance Liquid Chromatography Conditions

conditions of analysis	dicamba	picloram
mobile phase	methanol/water (50/50 v/v) and 0.005 M tetrabutylammonium phosphate	4% acetic acid in water/acetonitrile (95/5 v/v)
flow rate (mL/min)	1	1.5
wavelength (nm)	210	254
range of absorbance (AUI)	0.1	0.01
retention time (min)	7	17
width	5	20
attenuation	1	2
loop (μL)	20	100

picloram was according to the method of Cheng (1969) (Table 1). Fortified water samples used in the recovery studies were prepared by dissolving dicamba and picloram standards into separate water solutions. All soil samples used in this study were field–moist and passed through a 2.0-mm sieve.

Before dicamba was extracted from soils, samples were first subjected to ultrasonic vibration [25 g of moist soil with 80 mL of methanol/water solution (50:50 v/v) for 40 min]. Soil extracts were centrifuged, and a 20-mL aliquot was decanted and diluted to 100 mL with deionized–distilled water. Extraction of picloram from soils was accomplished by shaking 100 g of field-moist soil with 100 mL of 2 N KCl for 60 min. Soil extracts were centrifuged, and the supernatant was decanted. The supernatant solutions were analyzed according to the methods applied to water samples using SPE cartridges. The extraction process for removing dicamba and picloram from soil is outlined in more detail in Table 1.

SPE and HPLC Analysis. SPE cartridges were connected to 75-mL-capacity polypropylene sample reservoirs and placed on a vacuum manifold system using adapters. Cartridge sorbents were conditioned to activate the packing materials before the extraction of samples. Aminopropyl SPE cartridges were conditioned using 1 N acetic acid and distilled water; octadecyl SPE cartridges were conditioned using methanol and 4% acetic acid. Cartridges, after conditioning and before application of samples, were never allowed to dry; 1 mL of solution always remained above the cartridge resin. Water samples (50 mL) were transferred to the SPE cartridge reservoirs and eluted through the cartridges at a flow rate of approximately 2–5 mL min^{-1}. After samples containing picloram were eluted through the octadecyl SPE cartridges, the cartridges were washed with methanol; no post-treatment was necessary for octadecyl SPE cartridges. After the water samples were eluted, and the post-treatment in aminopropyl cartridges was completed, both types of cartridges were dried for 10–15 min using vacuum. The aminopropyl cartridges were rinsed with four portions of 500 μL of 0.1 N K_2HPO_4 and the octadecyl cartridges using two portions of 2 mL of 25% acetic acid in water (Table 2). We found that pesticide recovery was enhanced by about 10% when pesticides were eluted from the cartridges using several small aliquots as compared to one larger aliquot. Each aliquot remained in contact with the packing material for about 1 min. Dicamba and picloram were, respectively, collected in either 2- or 5-mL graduated glass vials, vortexed, and the volumes brought to 2 or 4 mL with the different mobile phases used for their HPLC analysis.

The mobile phase for dicamba analysis was 50/50 methanol/water with 0.005 M tetrabutylammonium phosphate at a flow rate of 1 mL/min. For picloram analysis, the mobile phase was 95/5 4% acetic acid in water/acetonitrile at a flow rate of 1.5 mL/min (Table 3). The mobile phase was never allowed to remain idle in the system; the columns were completely purged with acetonitrile or methanol every 24 h. The sample volume injected for dicamba analysis was 0.08 mL and for picloram analysis was 0.4 mL.

RESULTS AND DISCUSSION

Recently, pesticide extraction and concentration with SPE was used to extract dicamba and picloram from water and soil samples (Wells, 1986; Wells and Michael, 1987; Arjmand et al., 1988; Michael et al., 1989). The method proposed here for the analysis of dicamba in water was modified after that of Arjmand et al. (1988). We found that the strong nonpolar octadecyl C_{18} reversed-phase cartridges (500 mg) were not efficient for dicamba analysis. Previous results indicated the maximum percent recovery of dicamba from a 50 ppb water sample was only about 60–80%. In addition, Arjmand et al. (1988) recommended samples be acidified to approximately pH 1 before elution through octadecyl SPE cartridges. Our studies showed that, at this low pH, octadecyl material was stripped off the silica stationary phase along with dicamba, as the eluted solutions contained colloidal substances that interfered in the analysis of dicamba. Using aminopropyl SPE cartridges (500 mg) resulted in improved recoveries of dicamba over the octadecyl SPE cartridges.

Our method for the analysis of picloram in water samples was modified after that of Wells et al. (1984).

Table 4. Percentage Recovery of Dicamba and Picloram from Water and Soil Samples

pesticide added (ppb)	recovery			
	n^a	dicamba	n	picloram
water samples				
10	10	91.7 ± 22.0	8	84.9 ± 13.0
20	11	99.2 ± 13.2	20	90.7 ± 6.0
40	6	92.9 ± 10.0	6	92.3 ± 7.0
50	10	90.3 ± 5.0	10	91.4 ± 10.4
60	4	93.0 ± 3.5	4	96.4 ± 2.4
soil samples				
10	4	83.2 ± 5.6	4	88.3 ± 6.0
100			4	79.4 ± 10.5
200			4	86.2 ± 11.3
500	4	85.8 ± 10.5	4	88.9 ± 9.0

[a] Number of samples analyzed.

Table 5. Detection Limits and Efficiencies Using SPE and HPLC Analysis for Quantifying Dicamba and Picloram in Water and Soil Samples

pesticide	detection limit (ppb)	recovery (%)	ref
water samples			
dicamba	1	91	present study
	10	93	Arjmand et al. (1988)
picloram	8	91	present study
	2	92	Wells et al. (1984)
soil samples			
dicamba	10	82	present study
picloram	10	89	present study
	10	61	Wells et al. (1984)

Water samples were made up to 1 N NaCl and acidified to pH 2 before they were passed through octadecyl SPE cartridges. Adding NaCl increased the percent recovery of picloram in water samples by up to 30%.

Water samples containing 10–60 $\mu g\ L^{-1}$ of dicamba and picloram in 50 mL of water were passed through individual SPE cartridges; average recoveries are summarized in Table 4. The percent recovery using a 25-mL sample was the same as for 50 mL; however, the percent recovery was dependent upon sample concentration. The recovery of dicamba from water samples varied from 90.3 ± 5.0 to 99.2 ± 13.2% for samples containing 50 and 20 ppb of dicamba, respectively. Picloram recoveries from water samples ranged from 84.9 ± 13.0 to 96.4 ± 2.4% for 10 and 60 ppb of picloram, respectively. The detection limits for pesticides in water samples subjected to direct HPLC analysis were 25 ppb for dicamba and 100 ppb for picloram. SPE cartridges used in our method decreased detection limits by a factor of 25 times for dicamba and 12.5 times for picloram. Therefore, the detection limits of dicamba and picloram in water using SPE cartridges were lowered to 1 and 8 ppb, respectively.

Comparison of water sample results of the present study with those obtained by others indicates that for picloram we achieved a slightly higher detection limit with a comparable mean recovery and for dicamba a significantly lower detection limit with a comparable mean recovery (Table 5).

Octadecyl SPE cartridges from Supelco (Envi and Supelclean) and Burdick and Jackson were tested to determine which gave the best recovery of picloram. The results indicated that the recovery of 20 ppb picloram in water samples was only about 75 ± 10% (triplicate analyses) for the two Supelco octadecyl SPE cartridges. Aminopropyl cartridges from J. T. Baker and from Burdick and Jackson were tested using a 500 ppb dicamba water sample. Approximately 10–20% greater recoveries were obtained when using the J. T. Baker

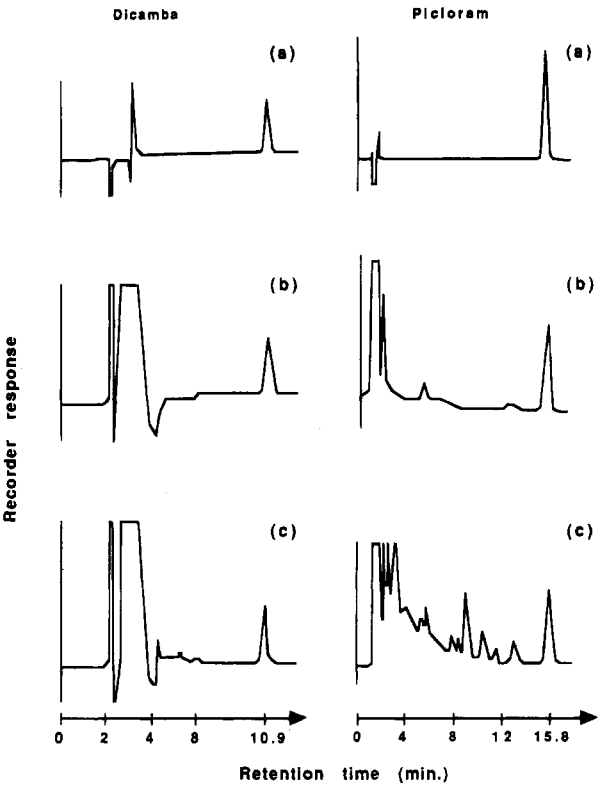

Figure 1. Examples of chromatographs for dicamba and picloram: (a) 500 ppb standards; (b) solutions that were passed through SPE cartridges; (c) solutions that were recovered from soil samples.

cartridges. We also found that the difference in percent recoveries of either dicamba and picloram varied between 10 and 20% among different lots of SPE cartridges from the same vendor.

Soils used for dicamba and picloram recovery studies contained 1.4% organic carbon and 23% clay, and had a pH of 8.0. In preliminary studies, soil samples for picloram analysis were extracted according to the methods of Cheng (1969) and Wells et al. (1984). We found that with the Wells et al. (1984) method, background noises interfered with the detection of picloram using the HPLC conditions listed in Table 3. The method proposed by Cheng (1969) was originally developed for use on samples containing from 1 to 25 ppm of picloram. We modified this method so that soils containing picloram at concentrations below 1 ppm could be analyzed.

Results of the recovery studies involving soils amended with either dicamba or picloram indicated the detection limit and mean recovery were as good as or better than those of previous studies using SPE techniques (Table 5). The detection limit for both dicamba and picloram extracted from soils was approximately 10 ppb; for picloram, comparable recoveries were obtained for soil samples fortified with 10 and 500 ppb of picloram solutions. Examples of chromatographs of dicamba and picloram standards, solutions passed through SPE cartridges, and solutions recovered from fortified soil samples are presented in Figure 1.

In summary, the SPE technique with HPLC analysis reported here is capable of measuring picloram and dicamba in water and soil samples at low parts per billion levels. The method is simple and much less time-consuming than liquid–liquid extraction and derivatization. Approximately 24 water samples can be analyzed daily, starting from SPE cartridge preparation

(using a 12-port vacuum manifold) to HPLC analysis. Extraction of dicamba or picloram from 24 soil samples takes approximately 8 h, after which another day is required for HPLC analysis. Once the procedure is learned, a laboratory assistant should be able to process and analyze approximately 120 water samples or 50 soil samples for dicamba or picloram weekly. The estimated cost for cartridges and HPLC grade chemicals required to analyze 24 water samples is about $75.

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