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Determination of Picloram in Soil and Water by Reversed-Phase Liquid Chromatography

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Abstract. A reversed-phase liquid chromatographic method is presented for the determination of picloram in the parts per billion[2] (ppb) range in soil, soil solution, and stream samples. Quantification is effected by UV absorption at 254 nm. Derivatization is not necessary. The method permits 92% ± 7.1 recovery from water samples and 61.8% ± 11.1 recovery from soil samples.

Picloram (4-amino-3,5,6-trichloropicolinic acid) is a herbicide used for the control of woody and broadleaf plants and is marketed by the Dow Chemical Company[3] in formulations bearing the trade name Tordon®. Many agriculturally-important broadleaf crops are susceptible to very low levels of picloram (Ragab 1975; Thomson 1978). The potassium salt of picloram is very soluble in water and is slowly degraded by soil microorganisms (Hilton 1974). Therefore, the environmental impact downstream from the treatment of forested areas with picloram is of concern (Neary *et al.* 1979).

Methods of picloram analysis were reviewed (Zhemchuzhin 1978). Techniques utilizing pulse polarography (Whittaker and Osteryoung 1980) and high performance liquid chromatography (Skelly *et al.* 1976, Stevens 1979), suitable for the analysis of commercial formulations without derivatization, have been developed. Existing gas chromatographic (GC) methods for the analysis of picloram in water (Anon. 1968; Anon. 1973; Baur *et al.* 1972) and soil (Bjerke 1973; Ragab 1975) involve lengthy clean-up procedures followed by derivatization and analysis as the methyl ester.

Studies designed to quantify the off-site movement of picloram resulted in large numbers of soil and water samples for analysis, and a rapid, economical, and reliable procedure was desirable. A method applicable to biological samples was necessary, which minimized the health and explosive hazards of diazomethane used in the analysis by GC. This study was designed to establish a clean-up procedure for environmental soil and water samples sufficient to permit the reversed-phase liquid chromatographic (RPLC) analysis of underivatized picloram with UV detection.

Experimental

Apparatus

The liquid chromatograph was a Waters (Milford, MA) Model 710B intelligent sample processor and Model 6000A solvent pump, a guard column (7 cm × 2.1 cm I.D.) dry packed with Whatman (Clifton, NJ) CO:PELL ODS (30–38 μm), an Ultrasphere ODS (5 μm, spherical porous particle) analytical column (1UE732N or 1UE747N) obtained from Altex Scientific, Inc. (Berkeley, CA), a Waters Model 440 UV absorbance detector, and a Houston Instrument (Austin, TX) recorder. Hypodermic syringes with Luer Lok tips were purchased from Becton-Dickinson (Rutherford, NJ). A low-form Filtrator and a Model 190 sample concentrator were obtained from Fisher Scientific (Pittsburgh, PA). A Beckman (Irvine, CA) Model J2-21 centrifuge was used. A Mortar-Pestle Grinder with an agate mortar and pestle was purchased from Brinkmann (Westbury, NY). Silane-treated glass wool was obtained from Supelco, Inc. (Bellefonte, PA), and Econo-column Polypropylene columns (0.8 cm I.D.) were obtained from Bio-Rad Laboratories (Rockville Center, NY).

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[2] American equivalent 10^{-9}

[3] The use of trade and corporation names does not constitute endorsement by the USDA, but is provided as a reference

Reagents and Chemicals

Spectrophotometric *grade methanol* (MeOH) *and* acetonitrile (ACN) were obtained from Burdick and Jackson Laboratories, Inc. (Muskegon, MI). Sulfuric acid. potassium chloride, and spectrophotometric grade glacial acetic acid (HOAc) were obtained from J. 'I'. Baker Chemical Co. (Phillipsburg, NJ). Diethyl ether and spectrophotometric grade acetone were purchased from Manufacturing Chemists. Inc. (Cincinnati, OH). Ammonium hydroxide, potassium hydroxide, and potassium permanganate were obtained from Fisher Scientific (Pittsburgh, PA), sodium hydrogen sulfite from Alfa Products (Danvers, MA), and anhydrous sodium sulfate from Sigma Chemical (St. Louis, MO). Basic alumina. activity grade I, was obtained from Woelm Pharma GmbH and Company (Eschwege, Germany). Picloram analytical standard (99.0%) was obtained from the Dow Chemical Company (Midland, MI,. SEP-PAK C_{18} cartridges were purchased from Waters Associates, Inc. (Milford, MA).

Sampling

Stream water, soil, and soil solution (lysimeter) samples were collected from a treatment area of the Tuskegee National Forest, Tuskegee, Alabama, before and after application of picloram as Tordon 10K. The overall results of this study will be published elsewhere.

Sample Preparation

Soil solution samples were monitored at 20 ppb (20 μg/L) by direct HPLC injection of 50 μL of the samples as received. Liquid/liquid extraction was used for the determinations at lower concentrations. Samples not processed immediately were frozen until analyzed. Soil samples were air-dried, passed through a 2.0 mm sieve, and ground with the Mortar-Pestle Grinder.

Recovery

Water: Stream water samples were made 2N in KCl; the stream samples (800 mL) and soil solution samples (100 mL) were acidified with concentrated sulfuric acid to pH 2, and extracted 3 times with diethyl ether (1 × 150 mL, 2 × 50 mL for 800 mL samples, and 3 × 50 mL for 100 mL samples). The extracts were combined, and the organic solvent evaporated. The resulting samples were transferred to 4-dram vials with 4% acetic acid in ether and evaporated to dryness. Adsorption trapping on a reversed-phase sorbent (C_{18} SEP-PAK cartridges) was used as an additional clean-up procedure. The cartridges were prewashed with 5 mL ACN followed by 10 mL of 4% HOAc in water. One mL of 4% HOAc in water was added to the sample vial and was either allowed to stand overnight or maintained at 60°C for 1 hr. This solution was loaded onto the sorbent. Desorption was effected by 9.0 mL of 25% HOAc in water. Preparation of the cartridges and charging of the sample was accomplished with a side-arm filtering flask and house vacuum (530 mm Hg) to pull the solvents through the reversed-phase cartridges. A Fisher Filtrator was used to collect the eluted sample directly into a 10 mL volumetric flask. The samples were diluted to 10.0 mL. For stream samples, a 25 μL aliquot of the sample was analyzed by RPLC; for lysimeter samples, a 50 μL sample was injected into the HPLC.

For analysis of low volume lysimeter samples, a 9.6 mL aliquot was diluted to 10.0 mL with glacial acetic acid, and the sample passed directly through a two-cartridge tandem prewashed with 5 mL ACN and 10 mL of 4% HOAc in water. The sample was eluted in the same manner as previously described for a single cartridge.

Soil: Approximately 40 g of soil, prepared as described earlier, was accurately weighed (±0.01 g) into a centrifuge bottle and to which was added 80.0 mL of a 0.5 N KOH/10% KCl solution. The bottle was capped and placed in a boiling bath for 15 min. The sample was placed on a mechanical shaker for 15 min and centrifuged at 5900 g for 10 min. The aqueous solution was decanted into a graduated cylinder and the volume recorded. The aqueous extract was adjusted to pH 1 with sulfuric acid and centrifuged at 5900 g for five min to precipitate the humic acids. The aqueous extract was decanted into a 1-L polypropylene bottle, while the humic acid precipitate was washed with 20 mL of 0.1 N HCl, centrifuged at 5900 g for 5 min, and the decantate added to the aqueous extract. The humic acid precipitate was washed with ether (3 × 10 mL), and the ether washes were combined and transferred to a 500 mL evaporation flask. The aqueous extract was diluted with 700 mL of distilled water, made 2N in KCl, and readjusted to pH 1 with sulfuric acid. The solution was extracted 3 times with ether (1 × 150 mL, 2 × 50 mL). The ether extracts of the aqueous fraction and the humic acid ether washes were combined, evaporated to dryness, and redissolved in 20 mL ether. (The manufacturer's protocol (Bjerke 1973) presents an alternative method for the preceding steps if an explosion-proof centrifuge is available).

The sample was subjected to adsorption trapping on alumina. A column of 1.0 g of basic alumina (activity grade 1), topped with a plug of silanized glass wool and 0.5 g anhydrous sodium sulfate, was prepared in a Bio-Rad polypropylene column. Columns, with the supports removed, were assembled piggy-back fashion to prepare a suitable solvent reservoir. The column was prewashed with 20 mL of acetone followed by 20 mL of ether. The 20 mL of sample in ether were charged to the alumina column, and the column was washed with 20 mL of ether followed by 20 mL of acetone. The picloram was eluted with 20 mL of 10% ammonium hydroxide in methanol (v/v), and the eluate was evaporated in the sample concentrator until only the aqueous portion remained. Additional clean-up of the sample was effected by adding 2.5 mL of 6N H_2SO_4 and 0.5 mL of saturated aqueous $KMnO_4$ to the sample. The sample remained at room temperature for 5 min, after which 5M $NaHSO_3$ was added dropwise until the solution was colorless. The sample was extracted twice with 10 mL of ether. The ether extract was charged to a second alumina column prepared and eluted identically to the first. The collected eluate was evaporated to dryness using the sample concentrator. One mL of 4% HOAc in water was added and the sample adsorbed on a SEP-PAK C_{18} cartridge as described in the recovery section for water samples. A 50 μL injection of the eluate from this procedure was analyzed by RPLC.

For the determination of recovery levels from soil, air-dried soil was fortified by the addition of enough aqueous picloram solution to bring it to 75% of field capacity (Cotterill 1980). The samples were placed in a mechanical shaker for 30 min, allowed to stand for 48 hr, air-dried, and extracted as described above after storage for three months at room temperature.

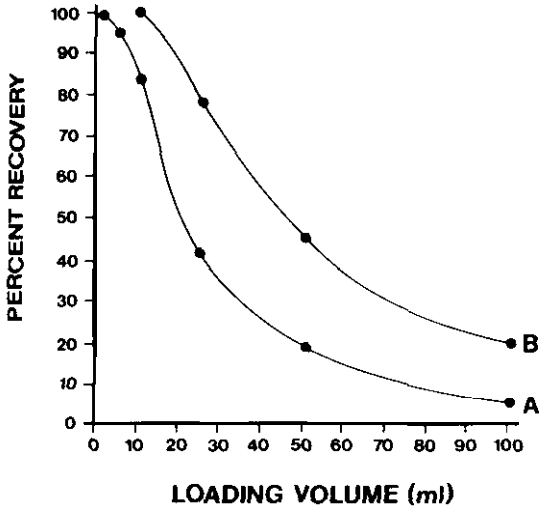

Fig. 1. Percent recovery of picloram as a function of sample loading volume through a single cartridge (A) or a two-cartridge tandem (B) system

Reversed-phase Liquid Chromatographic Analysis

The HPLC mobile phase consisted of 4% acetic acid/acetonitrile (95:5), (v/v), at a flow rate of 1.5 mL/min. The ultraviolet detector was operated at 254 nm and 0.005 a.u.f.s. The system was completely purged with acetonitrile every 24 hr. The mobile phase was never allowed to remain idle in the system.

Quantitation

Determinations of retention times and integrations of peak areas were performed by a Perkin-Elmer Model Sigma 10B data handling system (Norwalk, CT), and a linear detector response was established. A minimum of two injections was made for each sample. An average response factor was calculated, based on standard injections made every fourth sample, for those injections made within a given mobile phase batch. Good batch-to-batch agreement was observed.

Results and Discussion

Traditional methods for extracting and concentrating organic compounds from aqueous solution mainly involve liquid/liquid extractions which are costly in terms of labor, the high-purity solvents required, and the disposal of spent solvent. An alternative is to use adsorption trapping (Ogan *et al.* 1978) which involves adsorbing the compound of interest from an aqueous sample onto a solid sorbent. Various adsorbents have been used and adsorption trapping can precede analysis by either GC or LC.

Previous attempts to clean extracts of samples containing picloram with Florisil®, magnesia-Celite, alumina, and silica gel reportedly failed (Saha and Gadallah 1967) due to strong adsorption on these materials. Chromatography on basic alumina has been successfully utilized to remove extracted contaminants from soil samples containing picloram (Bjerke 1973).

Recently, reversed-phase material has become popular as a potentially useful sorbent for adsorption trapping. Several manufacturers now offer reversed-phases in convenient-to-use disposable cartridges for this purpose. Reports (Bushway 1981; Beier and Greenblatt 1981) of quantitative recoveries of organic compounds by reversed-phase adsorption trapping from aqueous samples of up lo one liter encouraged attempts to effect the trace enrichment on SEP-PAK C_{18} cartridges of large volume water samples containing picloram.

Samples containing 4.3 μg of picloram in 1.0, 5.0, *10.0, 25.0,* 50.0, and 100.0 mL of 4% acetic acid in water were prepared in triplicate and passed through individual SEP-PAK C_{18} cartridges. The average recovery is plotted as a function of loading volume in Figure 1, curve A. Recovery was strongly dependent upon loading volume and quickly dropped below 50% at 25 mL. Similar non-linear dependencies of extraction efficiency upon sample loading volume have been shown (Sanar *et al.* 1979; and Nyagah 1981). Extraction efficiency improved when a two-cartridge tandem was used for trapping the sample (Figure 1, curve B). The observed recovery from a C_{18} bonded phase is a result of complex interactions dependent upon the chemical nature of picloram and the hydrophobic and silanophilic interactions with the sorbent (Wells 1982).

Based on Figure 1, adsorption trapping on reversed-phase sorbent is deemed unsuitable for large volume samples, but 1.0 mL and 10.0 mL samples can bc quantitatively recovered from a single cartridge or a two-cartridge tandem, respectively. For stream samples (800 mL) and lysimcter samples (100 mL), an initial concentration step by liquid/liquid extraction was used prior to reconstitution in 1.O mL of 4% HOAc in water and passage through a single cartridge. For lysimeter samples, the number of required extractions was reduced by monitoring new samples by direct injection into the HPLC system, or by passage of a IO mL aliquot through a two-cartridge tandem prior to HPLC analysis. Chromatograms of picloram standard, and typical lysimcter and stream samples, are illustrated in Figure 2.

The clean-up procedures for soil samples involved combinations of liquid/liquid extraction and adsorption trapping on both basic alumina and reversed-phase sorbents. The soil extraction procedure for picloram was patterned after that reported

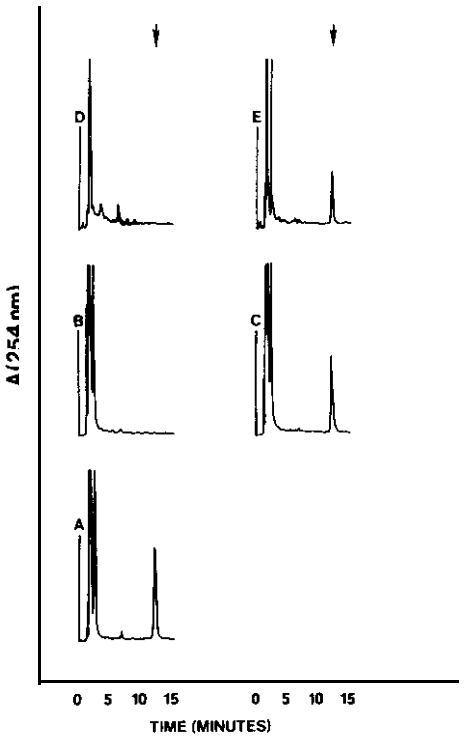

Fig. 2. Chromatogram of picloram standard, 30 ng (A). Typical chromatograms of lysimeter samples showing the absence (B) and presence (C) of picloram. Chromatograms of samples representative of the control stream (D) and stream samples collected downstream from the treated area (E)

by the manufacturer. However, notable changes have been made to adapt the procedure to our equipment and/or to improve efficiency. The humic acid fraction was precipitated and treated separately. Anhydrous sodium sulfate was added to the alumina columns, and the sample loading volume was reduced from 50 mL to 20 mL. The column diameter was 0.X cm. An additional adsorption trapping step on reversed-phase sorbent was added and derivatization is unnecessary as the mode of analysis differs (HPLC *vs* GC).

For soil samples, the apparent ppb determined by comparison with external standards was adjusted to the ppb of a 40-g sample on a dry weight basis:

$$\text{ppb} = (\text{apparent ppb}) \times \frac{\text{liquid added} + (\text{sample weight} \times \text{moisture content})}{\text{liquid recovered}} \times \frac{40}{\text{sample weight} (\text{sample weight} \times \text{moisture content})} \times \frac{100}{\text{average \% recovery}}$$

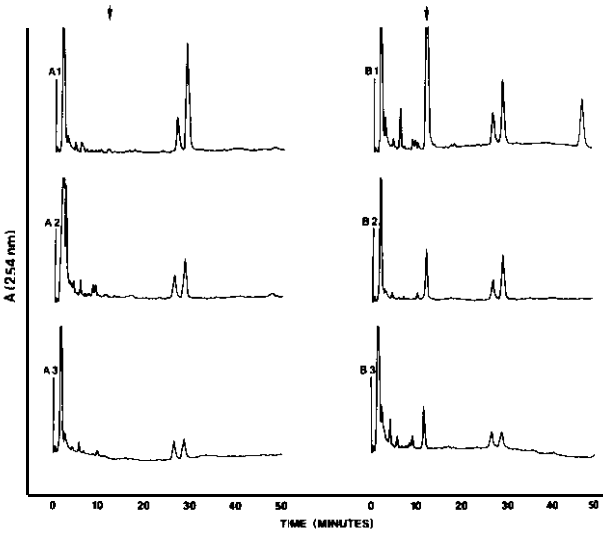

Fig. 3. Chromatograms of soil samples from two sampling dates (A = pretreatment, B = post-treatment) taken at three sampling depths: (1) 0–6″, (2) 6–12″, and (3) 12–18″

where liquid volumes are expressed in milliliters. weight in grams, and the moisture content is percent moisture divided by 100.

Figure 3 presents chromatograms of soil samples collected after treatment with a pelleted picloram formulation prior to and following rainfall. The chromatograms also illustrate the change in extractable organic contaminants as a function of sampling depth.

The average recovery of picloram (± s.d.) by liquid/liquid extraction was dctcrmincd from fortification experiments to be 92.0% ± 7.1 for both stream and lysimeter samples and 61.8% ± 11. I for soil samples (Table I). The detectability limits were established as 0.5 ppb for stream samples (800 mL), 2.0 ppb for lysimeter samples (100 mL), and 10.0 ppb for soil samples (40 g).

Conclusions

The use of high performance liquid chromatography to analyze environmental samples for picloram has several advantages over existing gas chromatographic methods. First, it is possible to monitor lysimeter samples at 20 ppb by direct injection of the sample as received. Those lysimctcr samples that do not exhibit the presence of picloram at this level can then be extracted by the method described herein to I-educe the detectability limit to 2 ppb. This method effectively reduces the overall number of extractions that must be performed, especially for those samples taken soon after the treatment

'fable 1. Efficiency of the extraction procedure as determined for fortified soil and water samples

Sample	Fortification	Recovery	Sample	Fortification	Recovery	Sample	Fortification	Recovery
	(ppb)	(%)		(ppb)	(%)		(ppb)	(%)
Water			Water			Soil		
(100 mL)	5."	100	(800 mL)	1.0	80	(40 g)	15.0	50.7
		102			100			82.0
		88			110			
	49.9	93.8		25.0	89.6		25.1	64.1
		84.6			91.2			74.9
		90.0			94.4			53.4
	99.8	87.2		loo.6	88.4		250.8	56.4
		91.4			88.9			55.7
		90.8			86.6			57.2
	Average	92.0		Average	92.1		Average	61.8

when sampling rates are more frequent and levels of picloram are higher.

Second, reversed-phase adsorption trapping is an effective sample clean-up procedure and could be used in an extraction scheme prior to analysis by LC or GC. The procedure appears to increase the longevity of the analytical LC column because it is also composed of a reversed-phase bonded sorbent.

Third, derivatization of picloram by diazomethane is avoided because the compound is detected by ultraviolet absorbance as the underivatized free acid.

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