Rapid Analysis of 2,4-D in Soil Samples by Modified Soxhlet Apparatus Using HPLC with UV Detection

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Abstract

The 2,4-dichlorophenoxy acetic acid (2,4-D) is used as a systemic herbicide to control broadleaf weeds in wheat, corn, range land/pasture land, sorghum, and barley. In this study, a fast and efficient method is developed by selection of modified extraction apparatus and high-performance liquid chromatography (HPLC)–UV conditions for the determination of 2,4-D in soil samples. The method is applied to the study of soil samples collected from the agricultural field. The herbicide is extracted from soil samples by acetonitrile in a modified Soxhlet apparatus. The advantages of the apparatus are that it uses small volume of organic solvent, reduced time of extraction, and better recovery of the analyte. The extract is filtered using a very fine microfiber paper. The total extract is concentrated in a rotatory evaporator, dried under ultrahigh pure N_2, and finally reconstituted in 1 mL of acetonitrile. HPLC–UV at 228 nm is used for analysis. The herbicide is identified and quantitated using the HPLC system. The method is validated by the analysis of spiked soil samples. Recoveries obtained varied from 85% to 100% for spiked soil samples. The limit of quantitation (LOQ) and the limit of detection (LOD) are 0.010 and 0.005 parts per million (ppm), respectively, for spiked soil samples. The LOQ and LOD are 0.006 and 0.003 ppm for unspiked soil samples. The measured concentrations of 2,4-D in spiked soil samples are between 0.010 and 0.020 ppm with an average of 0.016 $\pm$ 0.003 ppm. For unspiked soil samples it is between 0.006 ppm and 0.012 ppm with an average of 0.009 $\pm$ 0.002 ppm. The measured concentrations of 2,4-D in soil samples are generally low and do not exceed the regulatory agencies guidelines.

Introduction

2,4-Dichlorophenoxy acetic acid (2,4-D) is a herbicide used on a number of crops. Herbicides derived from 2,4-D are commonly used as selective herbicides because of their high toxicity to dicotyledonous plants and relative atoxicity to monocotyledonous plants. In addition to their use as herbicides, these compounds also find use in high-input farming as germination promoters for the production of seedless fruits (1), defoliating agents, and generally as hormone growth regulators. Herbicides derived from 2,4-D are increasingly being used as an aquatic herbicides, in which they are advantageous because of short persistence time, relatively low cost, and known dynamics in aquatic environments. 2,4-D is itself a foliarly applied, translocated herbicide. Its mode of action is as a persistent auxin, the concentration of which does not fluctuate in response to stimuli in a way that is characteristic to natural auxins. The primary effect of 2,4-D on susceptible plants is to cause aberrant growth of young, rapidly growing tissues near the meristem, resulting in nutrient depletion followed by death.

For a long time, herbicides were considered to be innocuous to animals. However, 2,4-D has been associated with polychlorinated dibenzodioxins, particularly 2,3,7,8-tetrachloro-*p*-dibenzodioxins. This compound is a well-known toxicant, teratogen, and carcinogenic agent (2). Mounting toxicological evidence points to the fact that the herbicide itself may be associated with cytotoxic effects on animals. These concerns create an impetus to study the dynamics of this important herbicide after application in the environment. Because of the relative residual times of most herbicides and how and where they are applied, soil is the largest temporary reservoir of accumulation in the environment. Upon application of a pesticide, soil undergoes one or more of the following physical, chemical, or biological transformations: photodecomposition, volatization adsorption inactivation, leaching, chemical break down, microbial degradation, plant uptake, or sheet erosion.

In 1974, the Unites States Congress passed the Safe Drinking Water Act. This law requires the Environmental Protection Agency (EPA) to determine safe levels of chemicals in drinking water that may or do cause health problems. These nonenforceable levels, based solely on possible health risks and exposure, are called "maximum contaminant level goals" (MCLGs). The MCLG for 2,4-D has been set at 70 parts per billion (ppb) because EPA believes this level of protection would not cause any of the potential health problems described below. Based on this MCLG, EPA has set an enforceable standard called a "maximum contaminant level" (MCL). MCLs are set as close to the MCLGs as possible,

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considering the ability of public water systems to detect and remove contaminants using suitable treatment technologies. The MCL has been set at 70 ppb because EPA believes, given present technology and resources, this is the lowest level at which water systems can reasonably be required to remove this contaminant should it occur in drinking water. Most of the available information on 2,4-D levels in the environment has been reviewed in detail (3–7). Figure 1 illustrates structure of 2,4-D (CAS No.94-75-7, chemical formula $C_8H_6Cl_2O_3$, and its molecular weight of 221.04 g/mol (8).

Detecting how much of these herbicides runoff into streams is important because of their potentially harmful influence on the environment. Analysis of these acidic herbicides has traditionally been a difficult problem because gas chromatographic (GC) analysis of the compounds was hampered by the low volatility of the herbicides (in many cases requiring a derivatization step prior to analysis). Chromatographic techniques have found their greatest application in the determination and study of herbicide 2,4-D. However, application of chromatography involves lengthy clean-up procedures to separate interferents, a delicate derivatization step, and, sometimes, a second clean-up procedure to remove excess derivatizing agent. General comments on criteria for acceptable analytical methods and on other pertinent aspects of 2,4-D determination are reported in the literature (9–12).

At present, gas–liquid chromatography (GLC) with electron-capture detection (ECD) is the most commonly used and, generally, the most sensitive method (picogram level) for measuring 2,4-D residues. To improve the sensitivity of detection, the 2,4-D has to be transformed (derivatized), usually to a methyl ester by reacting with BF3-methanol, diazomethane, or with concentrated sulfuric acid-methanol; the first method may produce the best results. A recent review of derivatization methods and GLC columns for various substrates are given in the literature. High-pressure liquid chromatography (HPLC) is less sensitive than GLC–ECD (i.e., nanogram vs. picogram levels) but may be advantageous under some circumstances. Examination of the behavior of 2,4-D in soils has shown that organic matter, soil pH (surface horizons), and exchangeable aluminium (clay sub-horizons) are the key determinants for the percentage of 2,4-D adsorbed. As the adsorption/desorption process is the basic mechanism influencing herbicide availability, mobility, and degradation in soil, 2,4-D is likely to be more strongly bound in soils with a high content of organic matter than in those with a low content.

We have developed a method for the analysis of the 2,4-D herbicide using HPLC–UV. This technique is an improvement over the GC method because no derivatization step is necessary prior to analysis, and the method is comparatively faster than other HPLC techniques.

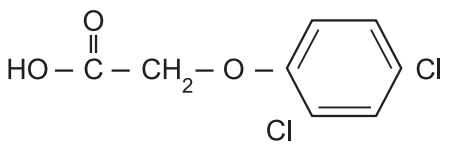

Figure 1. Chemical structure of 2,4-D.

Experimental

Quality assurance of sampling and sample pretreatment for herbicide determination in soils

In the study of soil pollution, importance has been given to sampling and sample pretreatment. Quality control procedures have been followed at each stage of the overall analysis.

A quality assurance program is a system of activities aimed at ensuring that information provided in environmental risk assessment meets the needs of the users of the data (13). Samples collected were as representative as possible of the soil to be characterized, and every precaution has been taken during sampling, pretreatment, and storage to ensure that samples remain unaltered. In environmental assessment studies this can be especially important because the results will be used to decide if corrective actions should be taken. Furthermore, standard operation procedures were written—to be followed in performing any operation—including objectives, sampling plans, preparation of containers and equipment, maintenance, calibration and cleaning of field equipment, sample preservation, packaging and shipping, health and safety protocols, and chain-of-custody protocols. Two main sampling approaches were considered: non-probability (judgment sampling) and probability (random and systematic sampling).

Many different soil samplers have been described in the literature, and the common principle is to preserve as much as possible the integrity of the sample. The most serious component is avoiding contamination of the sample by the main components of the materials used in the construction of the samplers. Porous ceramic, fritted glass, and new porous plastic materials were mainly used to avoid contamination. Careful cleaning of the sampling equipment and sample container was necessary to avoid cross contamination. Several cleaning procedures using detergents, steam cleaning, or high-pressure washing, followed by use of nitric acid and distilled water, were carried out as described in the literature (14). A chain-of-custody record has been completed containing all of the information related to sampling. This form included sample number (sample identification), signature of collector, date of sampling (year/month/day), site data, sampling techniques and description (depth sampled, boring, and drilling tools), conditions of transport and prestorage (material of container, duration, and temperature of transport), signature of persons involved in chain of possession, and inclusive dates of possession. Sample storage was performed carefully and under the most stringently controlled conditions in such a way that the integrity of the sample is preserved. Different systems were used to ensure the stability of soils during storage: low temperature storage (4°C), steam sterilization, freezing, or gamma irradiation. Low-temperature, short-term storage at 4°C was the method used to minimize microbial changes (15).

Sampling

Soil samples were collected in glass bottles at different sampling sites from agricultural fields sprayed with 2,4-D, after 15 days of the application. The samples were taken from the land surfaces up to 15-cm depth, after the removal of rests of leaves and roots from the soil surface. Three replicate (100 g) of soil samples

were collected and brought to the laboratory. The soil samples were also collected from the fields not exposed to the 2,4-D herbicide. The samples were then air-dried, homogenized, sieved, and refrigerated until the analysis. The soil samples were spiked with suitable volume (0.5 mL) of 2,4-D standard solution into 10.0 g of soil. The samples in the open bottles were allowed to stand for 24 h at ambient temperature before extraction.

Reagents and standards

The 2,4-D pesticide was procured from Dr. Ehrenstorfer (Augsburg, Germany). The chemicals used in this study were of HPLC grade. Acetonitrile, methanol, dichloromethane, and hexane were from Merck (Darmstadt, Germany). HPLC-grade water was obtained by purifying demineralized water in a Milli-Q system (Millipore, Bedford, MA). All other chemical compounds used in this study were procured from Sigma-Aldrich, Dr Ehrenstorfer, and Prochem. These were used for calibration of instrumentation, estimation of analytes, and validation of analytical methodology.

Sample extraction

The use of any extraction technique requires some external justification that the technique is capable of fulfilling its function (i.e., extraction of pollutants from soil). External justification for the use of extraction techniques is achieved through regulatory agencies such as the United States EPA and their equivalents in other countries, which provide the necessary information over many years that approve a particular technique. The EPA is regarded as one of the main sources of approved methods for extraction. EPA method 3540 (Soxhlet extraction) has been used as guidelines. In this study, a modified Soxhlet apparatus (Figure 2, courtesy of Mr. P.S. Kshirsagar, Glass Blowing Section, NEERI, Nagpur, India) is used to extract the herbicide analyte from the soil samples.

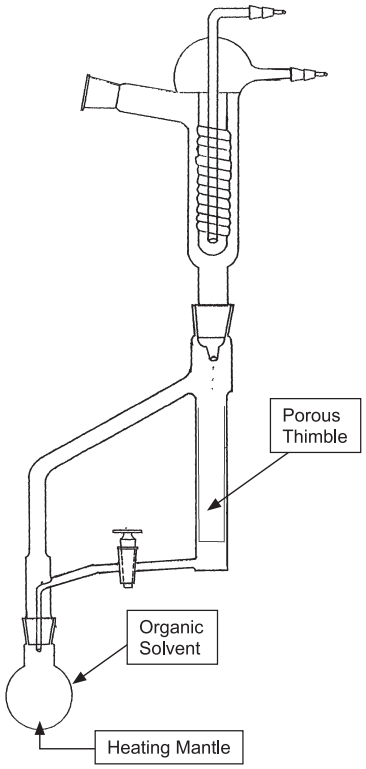

Figure 2. Modified soxhlet apparatus.

Instrumentation

Analytical HPLC was performed using on a Shimadzu LC-10AD system (Shimadzu, Kyoto, Japan) equipped with a Shimadzu SPD-M10A diode array detector. The collected data was processed using a LC workstation with CLASS LC-10 software. Compound (2,4-D) was analyzed on a Waters µBondapak C_{18} with (4.6-mm i.d. × 250-mm length, 5-µm particle size) column (Waters, Milford, MA) at flow rate of 1 mL and detector wavelength λ = 228 nm. The injection volume was 3 µL and Rheodyne (Rheodyne, Rohnert Park, CA) 7725i injection valve with a 20-µL sample loop was used. The mobile phase consisted of 75% acetonitrile (HPLC grade) (Merck, Darmstadt, Germany) and 25% H_2O (double distilled). Optimized isocratic elution was performed at 25°C, because gradient elution was not necessary. The HPLC chromatogram consisted of single peak of the standard analyte (2,4-D) (Figure 3). The spectrum of the analyte has been illustrated in Figure 4 .The column (stationary phase) was flushed for at least 20 min between the two consecutive injections. All of the solvents and solutions used in the mobile phase were previously filtered and degassed by ultrasonic application. Chromatographic conditions used in this study have been summarized in Table I.

In order to optimize the HPLC parameters, the conditions were monitored by varying the flow rates (mL/min) and the detector wavelength (λ). It was observed that the maximum absorbance of the analyte and best selectivity of the peak was at the detector wavelength of 228 nm and flow rate of 1 mL/min.

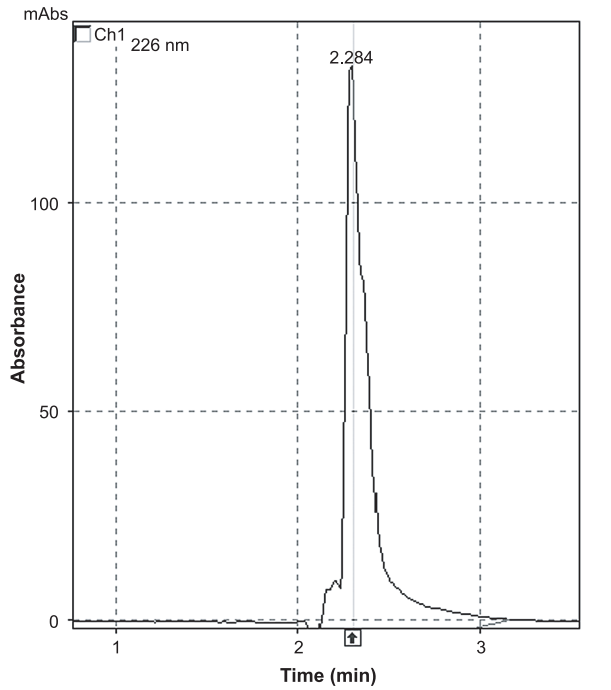

Figure 3. Chromatogram of 2,4-D herbicide obtained during analysis of soil sample.

The quantitative analysis was performed after the calibration curve was plotted with the standard having concentration between 0.001 and 0.009 ppm. Stock and working solutions of the compound were diluted in acetonitrile and refrigerated until use.

Analytical method

Methodology is based on the use of modified Soxhlet apparatus (Figure 2) for fast and efficient extraction of the analytes from the soil samples and LC with UV detection at 228 nm for the instrumental analysis of extracted soil samples. Different samples of soils were selected as matrices. The method developed included the selection of suitable extraction apparatus, clean-up procedure, and suitable extraction solvent and optimized HPLC conditions. The analytical method was validated by spiking a 10-g soil sample with 0.5 mL of 10-ppm standard herbicide 2,4-D and determining the recovery and precision. The spiked soil sample (10 g of soil sample spiked with 0.5 mL of 10-ppm standard 2,4-D herbicide) was stored in the porous thimble (cellulose). Approximately 25 mL of acetonitrile was stored in the bottom flask and heated by the isomantle. Clean up of samples was performed using a microcolumn of florisil. The final extract was then evaporated to 1 mL in a rotary evaporator, dried under UHP N_2, and reconstituted with acetonitrile (1 mL) (Figure 5). The same replicate samples were analyzed on a standard Soxhlet apparatus. Weighted regression of the recovery data showed for most analyte–matrix combinations, for spiked samples, that the method provides overall recoveries between 85% and 100% with standard deviations (SDs) of the reproducibilities less than 6%.

HPLC–UV in combination with the proposed modified Soxhlet apparatus allows for the determination of herbicide at concentration of at least the ppb level with recoveries greater than 90%, which comply with tolerance levels typically encountered today for individual herbicide.

Identification of the target compound 2-4 D, eluting from HPLC analytical column, was achieved through the combination of a retention time and background subtraction experimental UV reference spectra of the known 2,4-D standard.

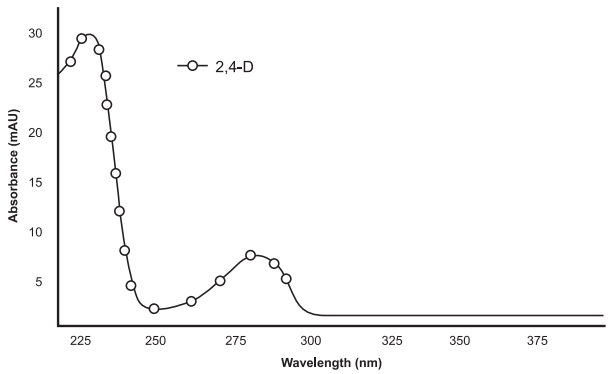

Figure 4. UV spectra of the peak observed in standard solution of 2, 4-D herbicide.

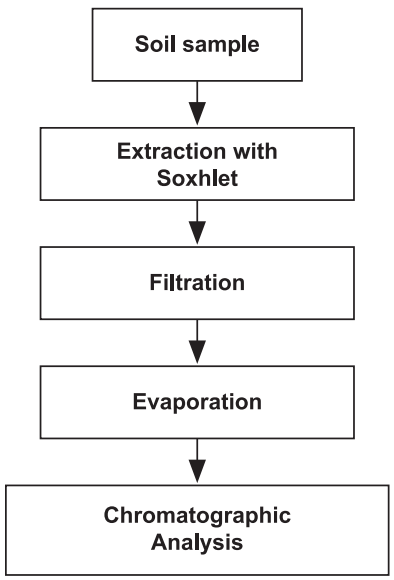

Figure 5. General schematic for analysis of 2,4-D herbicide by HPLC.

Table I. Chromatographic Conditions (HPLC–UV) Used for Detection of 2,4-D

Parameters	Details
Mobile phase	ACN*–H_2O (75:25)
Isocratic mode	–
Flow rate	1 mL/min
Temperature	25°C
Injection volume	3 µL
Detection	228 nm

* ACN = acetonitrile.

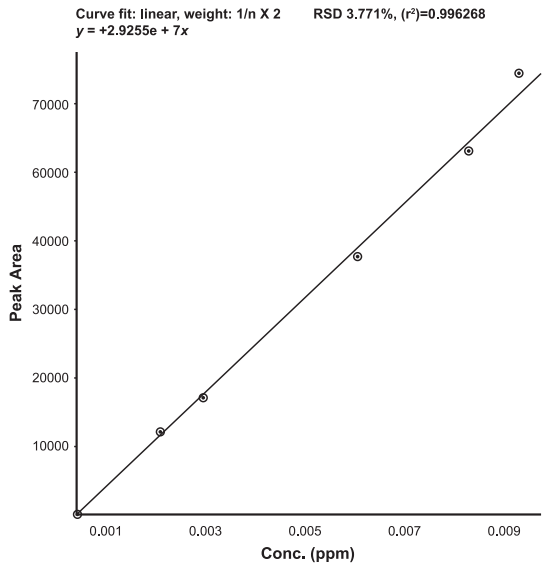

Figure 6. Calibration curve for 2,4-D.

Calibration

The process by which quantitative relation over a range of observed responses was established correlated each of several known concentrations to a corresponding signal and thus yielded a response curve. Using the calibration curve, the value of the unknown analyte could be determined. The herbicide quantitation was calculated from five-level calibration curve covering the range 0.001 to 0.009 ppm (Figure 6). The precision of the curve, as indicated by the relative standard deviation (RSD) was 3.771% and R^2 = 0.996268 for 2,4-D. The calibration curve data, limits of detection (LOD) and quantitation (LOQ) are presented in Table II.

Results and Discussion

The HPLC–UV techniques allow the accurate and reliable identification and quantitation of herbicide species, included in existing priority pollutant list at or below the levels established by international regulations.

The advantages of the modified Soxhlet apparatus were: small volume of organic solvent (25 mL), reduced time of extraction (20–25 min) when compared with the "traditional" way, and better recoveries (95% ± 5%). Table III presents the comparison between the standard and modified Soxhlet apparatus in different aspects.

The study reviewed here makes it clear that Soxhlet extraction with HPLC–UV procedures have improved the ease, reproducibility, and sample throughput for analysis of 2,4-D herbicide. Recovery with percent standard deviation (%SD) in spiked soil samples using standard Soxhlet and modified Soxhlet apparatus has been summarized in Table IV. Good analytical resolution and satisfactory precision expressed as SD was obtained. Based on the results, the new Soxhlet apparatus appears to represent a powerful tool in the field of sample extraction. It provides higher extraction efficiency than the popular classical Soxhlet, especially in extracting 2,4-D from soil. The results indicate that this method simplified the extraction, identification, and quantitation of the samples. It also significantly cuts the solvent waste and simplifies sample preparation, typically avoiding derivatization with reagents in current use, and provides high precision, as well as quality control. The herbicide identified and subsequently quantitated with HPLC–UV is summarized in the Tables V and VI. The measured concentrations of 2,4-D in spiked soil samples are between 0.010 and 0.020 ppm, with an average of 0.016 ± 0.003 ppm; and for unspiked soil samples it is between 0.006 ppm and 0.012 ppm, with an average of 0.009 ± 0.002 ppm.

Table II. Calibration Graphic Data, LOD, and LOQ (Unspiked Soil Samples)

	2,4-D
Calibration equation	y = +2.9255e + 7x
R^2	0.996268
LOD (ppm)	0.003
LOQ (ppm)	0.006

Table III. Soxhlet Extraction for the Determination of 2,4-D in Soil

	Classical Soxhlet (standard)	Modified Soxhlet apparatus (used in this procedure)
Process time (extraction time)	4 h	30 min
Further clean-up of soil extracts	SPE/Floricil	Floricil
Clean-up time	45 min	30 min
Concentration time	20 min	15 min
Overall analysis time	6 h	1.15 h
2,4-D recovery	55% ± 4%	95% ± 5%
SD	7.5%	3.771%
Detection limit	0.005 ppm	0.003 ppm
No. of cycles/h	4–5 cycles	Continuous drops (20–25 drops/min)
Solvent usage	250–500 mL (large solvent usage)	25–50 mL (small solvent usage)

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Table IV. Recovery Range and SD of 2,4-D Herbicide in Spiked Soil Sample

Soil sample (no.)	Overall percentage recovery ± %SD using modified Soxhlet apparatus	Overall percentage recovery ± %SD using standard Soxhlet apparatus
1	95% ± 5%	55% ± 5%
2	91% ± 2%	60% ± 4%
3	94% ± 5%	50% ± 4%
4	96% ± 1%	58% ± 6%
5	93% ± 4%	49% ± 6%
6	90% ± 2%	55% ± 5%
7	97% ± 1%	62% ± 5%
8	92% ± 5%	57% ± 6%
9	93% ± 4%	58% ± 4%
10	90% ± 5%	61% ± 4%
11	96% ± 2%	50% ± 3%
12	91% ± 5%	53% ± 6%
13	94% ± 4%	56% ± 7%
14	96% ± 2%	51% ± 6%
15	90% ± 4%	60% ± 5%
16	89% ± 1%	53% ± 2%
17	88% ± 2%	54% ± 4%
18	92% ± 5%	56% ± 4%
19	91% ± 5%	57% ± 3%
20	94% ± 4%	55% ± 3%

Table V. 2,4-D Residues Detected in Spiked Soil Samples

Soil sample (no.)	Concentration (ppm)
A1	0.012
A2	0.015
A3	0.010
A4	0.017
A5	0.018
A6	0.014
A7	0.019
A8	0.020
A9	0.018
A10	0.014
A11	0.017
A12	0.010
A13	0.016
A14	0.019
A15	0.013
A16	0.016
A17	0.019
A18	0.020
A19	0.014
A20	0.013

Table VI. 2,4-D Residues Detected in Unspiked Soil Samples

Soil sample	Concentration (ppm)
B1	0.007
B2	0.008
B3	0.006
B4	0.009
B5	0.010
B6	0.011
B7	0.008
B8	0.007
B9	0.006
B10	0.011
B11	0.012
B12	0.008
B13	0.006
B14	0.007
B15	0.009
B16	0.010
B17	0.012
B18	0.011
B19	0.008
B20	0.009

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