Bull. Environ. Contam. Toxicol. (1998) 60:371-378

Bulletin of Environmental Contamination and Toxicology

Methodology and Determination of 2,4-D and Triclopyr Residues Employing the GC-ITD in the Analysis of Lettuce Plants Cultivated in the Tala Valley, Republic of South Africa

A. Vogel

Technikon Northern Gauteng, Department of Chemistry, Private Bag X07, Pretoria North, 0116, Republic of South Africa

Received: 7 August 1997/Accepted: 24 December 1997

Both the 2,4-dichlorophenoxyacetic acid iso-octyl ester (2,4-D iso-octyl ester) and 2,4-dichlorophenoxyacetic acid dimethylamine salt (2,4-D dimethylamine salt) have been registered as hormone herbicides in the Republic of South Africa (Act 36 of 1947) for controlling the growth of annual broad-leaved weeds occurring in sugar cane plantations (Vermeulen and Grobler 1987). These hormone herbicides have been used extensively by the sugar cane farmers in the Tala Valley of Natal, South Africa. In many instances sugar cane and vegetable cultivation can be found on adjacent plots in the Tala Valley. Sugar cane plantations can also be found in areas surrounding the valley.

As vegetable plants grown in the Tala Valley depicted growth abnormalities leading to crop losses, a program was initiated in 1987 by the Plant Protection Research institute to monitor the occurrence of the hormone herbicides 3,6-dichloro-2-methoxybenzoicacid(dicamba),4-chloro-2-methylphenoxyaceticacid (MCPA), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2.4.5-T). 4-(4-chloro-2-methylphenoxybutanoic acid) (MCPB) and 4-(2,4-dichlorophenoxy-butanoic acid) (2,4-DB).

The 2,4-D acid (after hydrolysis) was found to be the most predominant residue (De Beer et al. 1989) detected in rain, water, dew and air sampled in the vegetable growing areas of the Tala Valley.

In the same monitoring period lettuce grown in the Tala Valley depicted similar growth abnormalities to lettuce plants exposed to 2,4-D iso-octyl ester under controlled laboratory conditions at the Plant Protection Research Institute.

Therefore the next step was to ascertain whether lettuce plants grown in the Tala Valley, exhibiting growth abnormalities, would be found to contain residues of 2,4-D. This would constitute an important part of the continued monitoring of 2,4-D, dicamba, MCPA, 2,4,5-T, triclopyr, MCPB and 2,4-DB occurring in rain, water, air and dew.

Experiments conducted by Breeze and Van Rensburg (1992), showed that 2,4-D phytotoxicity symptoms for lettuce develop at very low levels of 2,4-D iso-octyl ester exposer (426 ng per plant). It was therefore required to develop an analytical method where larger masses of the sample could be accommodated

in order to improve the chances of detecting 2,4-D, dicamba, MCPA, 2,4,5-T, MCPB and 2,4-DB. Furthermore, in order to optimise the detection of these hormone herbicides in lettuce, all forms of the hormone herbicides (bound or as esters) suspected of being in lettuce were converted to their acids by hydrolysis.

Detection and confirmation of 2,4-D, dicamba, MCPA, 2,4,5-T, 2,4-DB and MCPB was achieved by analysis of their 2,3,4,5,6 pentafluorobenzyl (PFB) ester derivatives using the gas chromatograph connected to an ion trap detector (GC-ITD).

Although the use of the [(3,5,6-trichloropyridinyl)oxy] butoxyethyl ester (triclopyr butoxyethyl ester) in the Tala Valley is not as extensive as that of the the 2,4-D esters, concern regarding its possible effects on lettuce plants necessitated the quantitative and qualitative analysis of its residues.

Both 2,4-D and triclopyr residue levels were monitored in lettuce plants obtained from three consecutive lettuce trials conducted by the Department of Horticulture, University of Natal, in the Tala Valley.

MATERIALS AND METHODS

All organic solvents were of high purity (Burdick and Jackson) and suitable for use in residue analysis.

The 2,4-D acid standard (Chem Service, West Chester, PA, USA) was recrystallised by dissolving approximately 0.5 g crude 2,4-D acid in 20 mL of methanol followed by the addition of 180 mL of distilled water. The solution was acidified by adding a few drops of concentrated hydrochloric acid ($pH \leq 1$) and the 2,4-D acid extracted out of the aqueous phase into (4 x 50 mL) dichloromethane. Excess hydrochloric acid in the dichloromethane extract was removed by gently rinsing with approximately 100 mL of distilled water. The volume of dichloromethane was reduced to approximately 20 mL and a few drops of carbontetrachloride added to obtain pure white crystals of 2,4-D acid. NMR analysis of the acid revealed a purity of 98.0%. Both the triclopyr acid standard (99.0%) and the 2,4,5-TP acid standard (99.5%) were purchased from Chem Service, West Chester, PA, USA and were not purified any further.

Identification and quantification of the PFB esters were performed using a Varian Model 3400 gas chromatograph coupled to a Finnigan Model 800 Ion Trap Detector Column (De Beer et al. 1989). The Varian Model 8100 autosampler was set to inject 2 µL of all samples employing a septum-equipped programmed injector (SPI). A DB-5 capillary column with a film thickness of 1 µm and i.d. of 30 m x 0.32 mm was used to separate the various hormone herbicides. The mean carrier gas had a velocity of between 24 and 25 cm per second at a temperature of 250 °C. Column temperature was 60 °C for 60 seconds, then programmed to reach 230 °C at a rate of 30 °C per minute (60-230 °C). The column temperature was maintained at 230 °C until the component of interest was eluted from the column. In order to elute all possible interfering impurities before injecting the next sample the column temperature was increased to 250 °C at a maximum rate per minute.

All lettuce samples analysed for hormone herbicides were picked specifically from vegetable growing areas in the Tala Valley and transferred in polyethylene bags to freezers set at -10°C. New freezers were specially used to avoid hormone herbicide contamination. Random samples, ideally sampled with the object of obtaining a total mass exceeding one kilogram were taken.

All lettuce samples were prepared in a hormone herbicide free environment and all equipment thoroughly cleaned and carefully handled in order to prevent contamination. The lettuce samples were cut into small pieces and kept frozen until analysed. All lettuce samples were analysed within a period of two weeks after cutting and freezing.

As hormone herbicide contamination of samples can occur during any stage of the analysis, reagent blanks were always included as part of the analysis. In instances where blanks were found to be contaminated with any one of the hormone herbicides, the source of contamination was identified and the analysis repeated.

A representative subsample of approximately 200 g was weighed into a 1 L beaker to which 10 mL (12.5 µg/250 mL) of the internal standard 2,4,5-TP was pipetted. 800 mL of a 0.1 N sodium hydroxide solution was added and the contents homogenized to a fine pulp using the Ultra Torrax. After stirring the contents vigorously for 30 minutes on a magnetic stirrer hotplate set between 60 and 65 °C (Sirons et al. 1982), 40 mL of concentrated hydrochloric acid was added to yield a pH of ≤ 1.

The cooled mixture (R.T.) was poured into a 2 L separatory funnel rinsing the beaker with a total of 200 mL distilled water in order to transfer the sample quantitatively. A total of 2 L of dichloromethane was used during the extraction process to extract as much of the hormone herbicides as possible from the aqueous phase in the funnel. After extraction, the volume of dichloromethane was reduced to 400 mL on a rotary evaporator set at a waterbath temperature of 40 °C. The dichloromethane extract was quantitatively transferred to a 1 L separatory funnel to which 100 mL of a 0.05 N sodium bicarbonate solution was added (Jensen and Glas 1978). After vigorous shaking for 2 minutes, the dichloromethane layer was poured into a second 1 L separatory funnel and the sodium bicarbonate layer into a third separatory funnel. The above sodium bicarbonate extraction procedure was repeated another three times.

The total 400 mL of sodium bicarbonate solution was gently rinsed with 100 mL dichloromethane which was discarded. The sodium bicarbonate extract was carefully acidified to a pH of ≤ 1 using concentrated hydrochloric acid and the hormone herbicides finally extracted into a total of 4 x 100 mL dichloromethane.

As described by De Beer et al. (1989), the volume of dichloromethane was reduced to approximately 10 mL (waterbath of rotary evaporator set at 40 °C) followed by the addition of 50 mL of toluene and evaporation to dryness (GC-ECD purposes). The extract was dissolved in acetone and quantitatively transferred to a 10 mL serum flask. The volume of acetone was reduced to 4 mL under nitrogen gas and 500 µL of a 5.0% v/v (in acetone) of 1 -bromomethyl-2,3,4,5,6-pentafluorobenzene solution and 200 µL of a 30.0% m/v potassium

carbonate solution added to the extract. This was followed by refluxing the sealed contents (teflon lined rubber seal) at 60 °C in a waterbath for three hours.

To a Pasteur capillary pipette containing a silanized glass wool plug, approximately 0.7 g of a 5.0% deactivated silica gel was added, followed by 0.2 g anhydrous sodium sulphate. 5 mL of hexane was poured over the column material to wet it, followed by the quantitative transferral of the sample using hexane (De Beer et al. 1989). The lettuce extract obtained from lettuce samples exceeding 100 g was divided into two portions and each passed through separate columns. In order to remove excess reagent the column was eluted with a total of 9 mL of 1:3 solution of toluene-hexane and the eluate discarded. The 2,4-D pentafluoro and triclopyr esters were eluted with a total of 10 mL of 1:3 hexane-toluene solution. The eluate was evaporated to about 1 mL using nitrogen gas. Hexane was used to quantitatively transfer the sample into a 2 mL auto-injector vial and the hexane evaporated to near dryness using nitrogen gas. 100 µL of hexane was added (calibrated Transfer pipette) to the sample extract and 2 µL injected onto the GC-ITD.

RESULTS AND DISCUSSION

Very sensitive methods are required when analysing for pesticide residues. In addition pesticide residues detected in environmental samples require confirmation. The gas chromatographic-electron capture (GC-ECD) system may be used for the purpose of partial confirmation (Jensen and Glas 1978). However as the gas chromatographic-ion trap detection (GC-ITD) technique enables the identification of compounds, this technique was chosen for both measuring and confirming 2,4-D as well as the other hormone herbicides in environmental samples (De Beer et al. 1989).

A completed validation of a pesticide residue method will include the determination of the recovery potential of the method with standard concentrations close to the level of the pesticide residue in the sample (Jensen and Glas 1978). Furthermore, a validated method must also include the confirmation and quantification of the residues to be analysed.

The method was validated for 2,4-D, triclopyr and 2,4,5-TP only. However, this method does allow for the detection and confirmation of dicamba, MCPA, MCPB, 2,4,5-T, and 2,4-DB since the gas chromatographic method adapted from De Beer et al. (1989) includes the detection and confirmation of these hormone herbicides.

Percent recoveries of 52.62% ± 8.54 for 2,4-D were obtained when 200 g homogenised lettuce plants (four replicates) were fortified with 500 ng standard 2,4-D acid and analysed using the method outlined. The internal standard was excluded and levels of recovered 2,4-D PFB measured against an external 2,4-D PFB ester standard. The large variation in the percent recovery of 2,4-D found, may be attributed to the variation in GC-ITD response due to the low concentration of the herbicides (Jensen and Glas 1978). Furthermore, differing amounts of non-polar constituents found in each sample, influences the thickness of the emulsions and hence the amount of residue extracted.

Therefore this justified the inclusion of 2,4,5-TP as an internal standard rather than employing external hormone herbicide standards.

Although the recovery values for 2,4-D may be regarded as low (Jensen and Glas 1978) it must viewed in the light of the low levels of herbicide residue being recovered (Jensen and Glas 1978).

Even though the percent recovery may be low, larger sample sizes will compensate for low recovery values. The extraction of a 200 g subsample containing 400 ng hormone herbicide will yield 200 ng equal to a theoretical 50% recovery. 50 g of the same sample will yield only 100 ng equal to a theoretical 100% recovery. Furthermore, 200 g samples were found not to significantly increase the levels of impurities extracted from lettuce (Figure 1).

Fortifying samples with a minimum of 500 ng 2,4,5-TP per 200 g subsample was necessary in order to detect all the ionic groups specially the parent ion.

Identification of each hormone herbicide was based on its corresponding retention time and its identity confirmed by detecting a minimum of four mass/charge values unique to that compound (Table 1).

Table 1. Ion groups of 2,4-D PFB, triclopyr PFB and 2,4,5-TP PFB used for detecting and confirming on the GC-ITD.

Hormone herbicide ester	M/Z_1	M/Z_2	M/Z_3	M/Z_4
2,4-D PFB	111	*175	181	400
Triclopyr PFB	146	181	*212	436
2,4,5-TP PFB	181	*196	223	448

* marks the ion used to obtain the appropriate integration value.

At the time of analysis the retention times were 19:31 minutes for 2,4-D PFB, 21:83 minutes for triclopyr PFB and 23:08 minutes for 2,4,5-TP PFB (Figure 1).

The 2,4-D acid standard and the 2,4,5-TP internal standard (both in the same solution) were converted to their PFB esters (De Beer et al. 1992). The ratio between 2,4-D PFB and 2,4,5-TP PFB was obtained by measuring the peak heights of both standards (GC-ITD) at equal concentrations. The ratio was then used to calculate the quantity of 2,4-D when detected in samples.

The reason for omitting the filtration step used by Allebone and Hamilton (1975) was that 200 g of homogenised lettuce samples, fortified with 500 ng 2,4-D, filtered and rinsed (500 mL of acidified water) using a sintered glass Buchner funnel, yielded low recoveries of 2,4-D (≤ 12%). Rinsing with 500 ml of acidified methanol yielded **a** recovery of ≤30%. Since the main problem is the retention of the 2,4-D by the homogenised plant material in the funnel, extracting the herbicide residues directly from the suspended homogenate seemed the obvious choice. In addition time was saved by eliminating the tedious process of having to brake the very thick emulsions resulting from the liquid-liquid extraction of the hormone herbicides from the filtered solution.

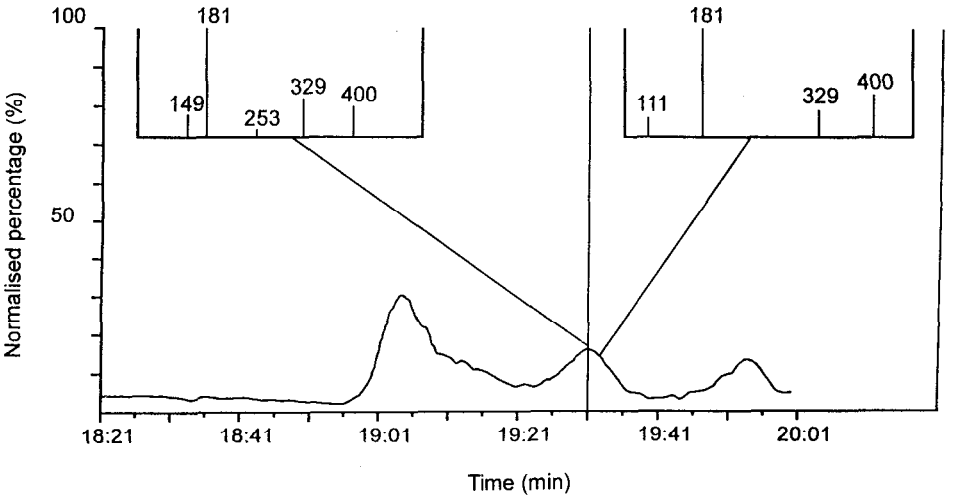

Figure 1. GC-ITD chromatogram of the summer lettuce extract (15/01/91) depicting the 2,4-D peak at 19:31 minutes, together with its mass to charge ratios.

The temperature found to be most suitable for the hydrolysis step was between 60 and 65°C (Sirons et al. 1982). Above 65°C, the impurity peak co-eluting with 2,4-D was found to dominate the 2,4-D peak.

Generally lettuce samples analysed did not exceed 200 g. This method is not recommended for the analysis of cabbage and pepper plants due to high levels of non-polar constituents present in these vegetables.

A minimum concentration of 0.5 ng per µL in hexane (2 µl injected) was required for reliable detection and confirmation of 2,4-D and triclopyr pentafluorobenzyl esters in the presence of plant impurities obtained from lettuce.

Preliminary analysis of lettuce plants randomly sampled in the Tala Valley revealed that the 05/12/89 samples contained 5.54µg 2,4-D per kg while 2,4-D could not be detected in the 30/01/90 and 19/03/90 samples.

Three lettuce trials (Department of Horticulture, University of Natal) were conducted in the Tala Valley starting in July 1990 and ending in February 1991. All samples obtained from these trials were analysed using the method outlined. The results are depicted in Table 2 (winter lettuce trial), Table 3 (spring lettuce trial) and Table 4 (summer lettuce trial).

Neither 2,4-D nor triclopyr were detected in lettuce obtained from the winter and spring trials (see Table 2 and 3).

Table 2. Results of the analysis of lettuce from the winter lettuce trial conducted in the Tala Valley.

Sampling date	2,4-D (µg/kg)	Triclopyr (µg/kg)
23/07/90	n.d.	n.d.
10/08/90	n.d.	n.d.
23/08/90	n.d.	n.d.
24/09/90	n.d.	n.d.

n.d. = not detected. Minimum detectable quantity for 2,4-D and triclopyr was 1 ng. 2,4,5-TP was detected in all samples.

Table 3. Results of the analysis of lettuce from the spring lettuce trial conducted in the Tala Valley.

Sampling date	2,4-D (µg/kg)	Triclopyr (µg/kg)
16/10/90	n.d.	n.d.
23/10/90	n.d.	n.d.
12/11/90	n.d.	n.d.

n.d. = not detected. Minimum detectable quantity for 2,4-D and triclopyr was 1 ng. 2,4,5-TP was detected in all samples.

2,4-D was detected in the January 1991 lettuce samples but not in the February samples (see Table 4 and Figure 1). 2,4-D was also detected in air samples taken over the January 1991 period (De Beer et al. 1992).

Table 4. Results of the analysis of lettuce from the summer lettuce trial conducted in the Tala Valley.

11/01/91	6.85	n.d.
15/01/91	3.66	n.d.
22/01/91	0.88	n.d.
05/02/91	n.d.	n.d.
22/02/91	n.d.	n.d.

n.d. = not detected. Minimum detectable quantity for 2,4-D and triclopyr was 1 ng. 2,4,5-TP was detected in all samples.

Observing the data in Table 4, it appears that the quantity of 2,4-D decreases as the plants grow to maturity. However, these results do not take into consideration the amount of 2,4-D taken up by the individual plants.

In Table 5 the approximate quantities of 2,4-D have been calculated using the average plant mass of 8 individual lettuce plants per sampling date.

It would be unwise to place too much significance on the accuracy of the quantities of 2,4-D calculated.

Table 5. Approximate quantities of 2,4-D per average individual lettuce plant.

Sampling date	2,4-D (μg/kg)	Approximate average plant mass (g)	Approximate 2,4-D per plant (ng)
11/01/91	6.85	2.77	19
15/01/91	3.66	6.46	24
22/01/91	0.88	32.01	28

However, what it does suggest is that 2,4-D levels do not decrease significantly as indicated by the data given in Table 4.

Lettuce samples picked on 05/02/91 weighed in excess of 800 g. The lack of detectability suggests dilution of 2,4-D by the large mass to an undetectable level, only when assuming that the quantity of 2,4-D within the plant had not changed.

Only lettuce plants grown in the Tala Valley during the summer (January 1991) contained detectable levels of 2,4-D while those grown in winter and spring did not. This trend is in agreement with the preliminary results obtained for lettuce (see under results and discussion) in which only the 05/12/89 sample contained detectable 2,4-D.

Acknowledgements. The author wishes to thank the Plant Protection Research Institute, Agricultural Research Council, Pretoria, as this work was conducted in their laboratories.

REFERENCES

Allebone JE, Hamilton RJ (1975) Determination of 2,4-D in plant tissue. J Chromatog 108 : 188-193

Breeze VG, Van Rensburg E (1992) Uptake of the herbicide [^{14}C] 2,4-D iso-octyl in the vapour phase by tomato and lettuce plants and some effects on growth and phytotoxicity. Ann appl Biol 120 : 493-500

De Beer PR, Sandmann ERIC, Van Dyk LP (1989) Gas-chromatography with ion trap detection to determine six herbicides in atmospheric samples. Analyst 114 : 1641-1645

De Beer PR, Smit CS, Van Dyk LP (1992) Air monitoring for pollution by auxin-type herbicides. Chemosphere 24 : 719-733

Jensen DJ, Glas RD (1978) Analysis for residues of acidic herbicides. In: Moye HA (edt.) Analysis of pesticide residues. John Wiley & Sons, Inc., pp 223-261

Sirons GJ, Anderson GW, Frank R, Ripley BD (1982) Persistence of hormone - type herbicide residue in tissue of susceptible crop plants. Weed Science 30 : 572-578

Vermeulen JB, Rankin J (1990) A guide to the use of herbicides. Twelfth Revised Edition. Department of Agriculture, Government Printer, Private Bag X 144, Pretoria, 0001, Republic of South Africa