Research Journal of Applied Sciences, Engineering and Technology 2(6): 592-595, 2010
ISSN: 2040-7467

Submitted Date: July 14, 2010 Accepted Date: August 15, 2010 Published Date: September 10, 2010

Gas Chromatographic Methodology for the Determination of Some Halogenated Pesticides

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Abstract: A Gas Chromatography (GC) methodology has been validated for the determination of some halogenated pesticides. Complete separation of the pesticide prepared in ethyl acetate was achieved on Rtx - 1 column with dimension, 30m x 0.25mm x 0.25µm. The GC equipped with electron capture detector was run using column temperature programmed from 80ºC (2 min) to 200ºC (15 min) at the rate of 4ºC/min giving a total analysis time of 47 min. The detector and injector were respectively at temperatures of 300 and 225ºC. The method was validated with respect to precision in terms of reproducibility of retention times and peak heights, linearity and minimum detectable quantity of the pesticides. Under the operated GC conditions, diuron eluted first while heptachlor epoxide was the last to elute. The chromatographic detector was more sensitive to α-endosulfan and β-endosulfan with Minimum Detectable Quantity (MDQ) of 0.002 ng. The detector was however, less sensitive to captan with MDQ of 0.08 ng. Margins of errors associated with the precision of the method in terms of reproducibility of retention times yielded standard deviation in the range of 0.026-0.063.

Key words: Chromatography, detected, linear range, methodology, pesticides

INTRODUCTION

Chemical pest control in Ghana has become increasingly important for cash crops such as cocoa as well as for food crops. In spite of increases in retail prices of pesticides, use of pesticides has increased drastically due to numerous pest problems facing the Ghanaian farmer (Gerken *et al*., 2001). Despite the positive impact of pesticides on food production, it is probably one of the most regulated chemical products used in the world. In Ghana Parliament had passed legislation, which regulates the use of pesticides and Environmental Protection Agency (EPA), the regulatory body, requires all importers and formulators of pesticides products to register with the Agency, and to request permission before importation and formulation (FAO programme, 1989). All these regulations are in place to help protect human health. Despite the many regulations, pesticide residues are unavoidably found in our food crops and the environment

When a pesticide product is applied on the field, the chemical is gradually lost as a result of breakdown, leaching and evaporation and the residue is the amount that remains after application (Cox, 1995). Whiles some pesticides have long residual activity and therefore persist in the environment, others have short residual activity and therefore do disappear from the environment or produce low residue concentration. It is therefore not surprising to find or detect residues of pesticides in the environment and food crops after usage. The Maximum Residue Level (MRL) is the maximum amount of the pesticide residue which if found in food substances will not cause any health effect or hazard (Cabtas and Martin, 1992). In International circles food crops with pesticide residue level above the stipulated MRL are likely to be rejected.

It is against this background that determination of pesticide residues in food and cash crops become very important. Pesticide residues determination in food crops allow us to know the quality of the food in terms of pesticide contamination. Chromatographic techniques such as GC, HPLC and TLC have been recommended for the determination of pesticides residues. Spectrophotometry could also be used for many pesticides, and calorimetric kits are available for choresterase inhibiting insecticides and fungicides (Afful, 2002; Lowor, 1999). Nowadays, hyphenated techniques such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) is becoming popular and fast gaining grounds for pesticide residues analysis (Balinova and Balinov, 2006). Gas Chromatography (GC) has traditionally, however, been used widely for analysis of pesticide residues in plants tissues, soils and water samples (Yeboah *et al*., 2003; Roseboom and Herbold, 1980).

GC consists of an injector, a column, a detector and a recorder or an electronic integrator. A few micro liters of the sample are injected into the injector. The sample is then vaporized in the injector and carried through the

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column by means of a carrier gas which serves as mobile phase. GC achieves separation of components in a sample by partition of the components between the mobile and stationary phases. The components are selectively retarded by the stationary phase as components interact with the stationary phase. This therefore causes the components to leave at different times depending on both the volatility of the compound and the affinity for the stationary phase. The time spent in the column is characteristic for each component under a specific set of operating conditions. This time is referred to as the retention time. As the components of a sample emerge from the column, the detector sends a signal proportional to the concentration of the component to the recorder. Each component registers as a peak on a chromatogram and is identified by means of the retention time and quantified by the peak area or peak height.

To a large extent, the choice of column determines the success of separation. The two types of columns used are packed and capillary. Packed columns are cheaper, easier to handle and are often used where high resolution is not required. Capillary columns are expensive but very good separation is achieved if handed correctly. The selection of column length depends on the required resolution and analysis time. Short columns (10-25 m) are useful for samples containing relatively smaller number of compounds. Intermediate column length of 25-30 m, provide sufficient separation power simultaneously with reasonable analysis time, are most cases used for many separations analysis (Kostiainen, 2000).

The success of GC in pesticide residue analysis is based on the sensitivity and selectivity of a wide range of detector systems. The critical properties of detectors are sensitivity, selectivity, linearity of response, reproducibility and reliability of operation (Kostiainen, 2000). Currently the Flame Ionization Detector (FID) is the most popular universal detector for pesticides analysis. It is mainly used for analysis of formulated products with few residues. Selective detectors, such as Flame Photometric Detector (FPD), Nitrogen- Phosphorus Detector (NPD), and Electron Capture Detector (ECD) are also available. Flame photometric detector is selective for determination of sulphur and phosphorus containing pesticides whiles Nitrogen - Phosphorus Detector (NPD) is selective for nitrogen and phosphorus containing pesticides. On the other hand, electron capture detector is selective for only halogenated pesticides, for example organochlorines pesticides (Yeboah, 2001).

In this study, a gas chromatographic methodology involving the use of Electron Capture Detector (ECD), Restek Rtx - 1 column (30m x 0.25mm x 0.25µm) with GC column programmed from 80ºC (2 min) to 200ºC (15 min), at the rate of 4ºC/min, injector 225ºC, and detector 300ºC has been validated for the determination of some halogenated pesticides. The GC methodology was validated by determining the precision of the method in terms of reproducibility of Retention Times (RT) and peak heights, linear range concentrations and Minimum Detectable Quantities (MDQ) of the pesticides. The manuscript is a research article.

MATERIALS AND METHODS

This study was carried out in 2008 at the Chemistry Department of Ghana Atomic Energy Commission.

Chemicals and reagents: The pesticides reference standards were obtained from Dr. Ehrenstorfeer Gmbh, Darmstadt. The purity of the pesticide standards ranges from 97- 99% and were used without further purification. Pesticide standards (1 mg/mL) were prepared by dissolving 0.1 g of the standard in 100 mL of ethyl acetate. These served as the stock solutions and were diluted to the desirable concentrations for analysis. The ethyl acetate used as solvent for the preparation of the pesticide standard solutions was of analytical grade and was obtained from BDH Limited in England.

Gas Chromatograph: The gas chromatograph was a Shimadzu 2010 series equipped with Electron Capture Detector (ECD), split/splitless injector, AOC-20s auto sampler and AOC-20i auto injector system. The column was Restek Rtx -1 capillary column with dimension 30 m x 0.25 mm x 0.25 µm. The carrier gas was nitrogen gas supplied from ANG 2381HC nitrogen - air generator with a column flow of 1.18 mL/min. The column temperature was programmed from 80ºC (2 min) to 200ºC (15 min) at the rate of 4ºC/min. The injector and detector temperatures were 225 and 300ºC, respectively.

Validation of the method: Precision of the method: Precision of the method was determined in terms of reproducibility of the retention time and the peak height for each pesticide. Four (4) replicates determination was carried out for each pesticide and 1 µL of 0.05 ng/µL of the pesticide standard was injected.

Minimum Detectable Quantity (MDQ): Minimum detectable quantity (MDQ) considered as the smallest quantity of the standard materials resulting in definite visible peak was determined for each of the pesticide by preparing and analyzing various pesticide standard concentrations in a range of 0.01-0.001 ng. The least pesticide concentration that resulted in visible peak was taken as the MDQ

Linear range: Linear range for the pesticides was determined by analyzing various calibration solutions in a concentration range of 0.02-0.25 ng for each pesticide and calibration curves for the linear range obtained by plotting the peak height against the corresponding concentration.

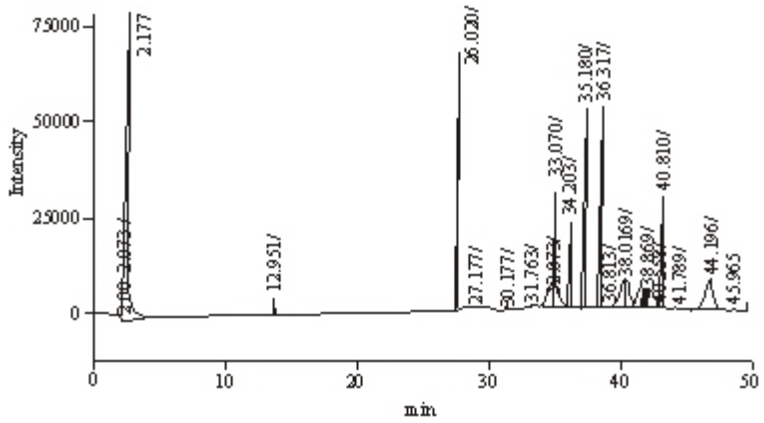

Fig. 1: Sample chromatogram of mixed standard of the nine pesticides with their retention times in minutes: diuron (12.951), lindane (26.020), aldrin (33.070), captan (34.203), triclopyr (35.180), α-endosulfan (36.317), dieldrin (38.009), β-endosulfan (40.810) and heptachlor epoxide (44.196)

Table 1: Retention times and corresponding peak heights

	Retention times/min						Peak height				
Pesticides	1	2	3	4	average	RRT(diuron)	1	2	3	4	average
Diuron	12.951	12.918	12.912	12.945	12.931±0.026	1.000	1044	1024	1028	998	1023.5±19.07
Lindane	25.956	26.020	25.970	26.012	25.989 ±0.032	2.009	4588	4554	4575	4560	4569.2±15.30
Aldrin	33.070	33.199	33.168	33.201	33.154±0.042	2.559	4600	4668	4703	4723	4643.5±64.42
Captan	34.203	34.071	34.108	34.104	34.121± 0.051	2.638	1124	1136	1098	1143	1124.7±19.47
Triclopyr	35.180	35.183	35.130	35.173	35.166±0.025	2.719	4547	4604	4613	4558	4580±32.85
Dieldrin	38.009	38.186	38.203	38.002	38.102±0.043	2.946	5537	5531	5532	5522	5530.5±6.27
α-endosulfan	36.317	36.315	36.152	36.155	36.234±0.063	2.802	8899	9242	8934	9062	9034±116.43
β-endosulfan	40.810	40.723	40.759	40.821	40.801±0.063	3.116	8753	9022	9113	8904	8948±85.02
Heptaclor epoxide	44.196	44.212	44.223	44.098	44.181±0.057	3.416	6828	6776	6943	6856	6850.2±68.42

Table 2: Data on calibration curves for the pesticides

Pesticides	Linear range concentration/ng	Regression equation	Coefficient of correlation (R^2)
Diuron	0.04–0.12	Y = 13801X–188.31	0.9939
Lindane	0.025–0.05	Y = 205415X+238.84	0.9967
Aldrin	0.06–0.20	Y = 71097X+790.92	0.9961
Captan	0.04–0.12	Y = 96152X–1484.3	0.9967
Triclopyr	0.04–0.12	Y = 794416X– 9329.5	0.9969
Dieldrin	0.05–0.30	Y = 27535X	0.9970
α-endosulfan	0.04–0.10	Y = 54963X+3978.5	0.9992
β-endosulfan	0.02–0.09	Y = 349114X+5112.6	0.9810
Heptachlor epoxide	0.02–0.15	Y = 112313X+1183.2	0.9997

RESULTS AND DISCUSSION

Chromatograms obtained for the pesticides are as shown in Fig. 1. The mixed standard which was analyzed contained nine halogenated pesticides, namely diuron, lindane, aldrin, captan, triclopyr, α-endosulfan, dieldrin, β-endosulfan and heptachlor epoxide. In all diuron eluted first with retention time of 12.951 min while heptachlor epoxide eluted at 44.196 min.

Table 1 shows the results of precision of the GC method in terms of reproducibility of the retention times and the corresponding peak heights. The Table 1 also shows the calculated relative retention times (RRT) using diuron as the reference chemical. Calculated RRT ranged from 1.000-3.416. Margins of errors associated with precision of the method in terms of reproducibility of retention time's yielded standard deviation in the range of 0.026 to 0.063.

Data on the linear range determination for the pesticides are presented in Table 2. The data contains the regression (calibration) equations for the determination of the pesticides, the linear range concentrations, and the coefficient of correlation (R^2) between concentrations and peak heights. The Coefficient of correlation between concentrations and corresponding peak heights ranges from 0.9810-0.9997. This is an indication of good correlation between the concentrations and the peak heights. In all, linear range concentrations were between 0.02 to 0.30 ng. The differences in the linear range for the pesticides are an indication of differences in detector response factors to the individual pesticides. Figure 2 is a sample calibration curve for the determination of dieldrin which is a plot of peak heights against the corresponding concentrations in the linear range.

The Minimum Detectable Quantity (MDQ), considered as the smallest quantity of the standard

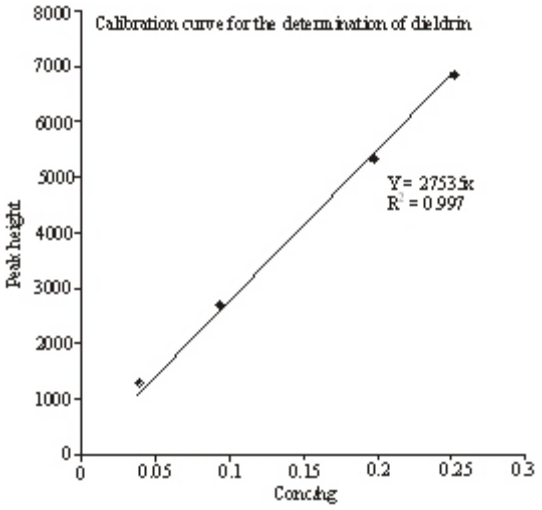

Fig. 2: Calibration curve for the determination of dieldrin

Table 3: Minimum detectable quantity (MDQ) for the herbicides

Pesticides	Minimum detectable quantities/ng
Diuron	0.05
Lindane	0.004
Aldrin	0.004
Captan	0.08
Triclopyr	0.008
Dieldrin	0.004
α-endosulfan	0.002
β-endosulfan	0.002
Heptachlor	0.0025

material resulting in a definite peak or chromatogram (Ambrus, 1998) obtained for the pesticides are presented in Table 3. MDQ was calculated as three times the baseline peak. The results in general suggest that the gas chromatographic detector (ECD) is sensitive for the determination of the pesticides. The results further suggest that the chromatographic detector used is more sensitive to the organochlorine pesticides namely, aldrin, dieldrin, α-endosulfan, β-endosulfan, heptachlor epoxide as the detector could response to small change in concentration of these chemicals (Table 3). With the organochlorine pesticides the detector shows more sensitivity to α-endosulfan and β-endosulfan compared to the other three. The detector was however, less sensitive to captan with MDQ of 0.08 ng as indicated in Table 3.

CONCLUSION

The gas chromatographic methodology validated is simple, fast and reliable and the Restek Rtx - 1 (30m x 0.25mm x 0.25μm) used as column gave a good separation of the nine halogenated pesticides administered in a mixed standard. Results obtained for minimum detectable quantity for the pesticides is an indication of sensitivity of the chromatographic detector for the quantitative determination of the pesticides investigated. The GC method can therefore be useful for routine analysis of halogenated pesticides.

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