Malaysian Journal of Chemistry, 2011, Vol. 13, No. 1, 057 – 062

Simultaneous Determination of Nine Commercial Pesticide Formulations by Gas Chromatography Multi-Pesticide with an Internal Standard Method

Chai Mee Kin[1] and Tan Guan Huat[2]

[1]*Dept. of Sc., Maths & Computing, College of Foundation & General Studies, Universiti Tenaga Nasional, Jalan IKRAM-UNITEN 43000 Kajang, Selangor, Malaysia*

[2]*Dept. of Chemistry, Faculty of Science, Universiti Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia*

Abstract : A gas chromatography multi-pesticide with an internal standard method has been developed to analyze simultaneously separate nine active ingredients (acephate, carbaryl, chlorpyrifos, chlorothalonil, diazinon, dimethoate, malathion, profenofos, quinalphos) in different commercial pesticide formulations. A mixture of pure standard solution spiked with 1-chloro-4-fluorobenzene as the internal standard was injected into the GC-ECD and a six point calibration curve that demonstrated a linear range was established for each target compound. Samples of each formulation, mixed with internal standard were analyzed five times to obtain coefficients of variation which are less than 1%. Three concentration levels of each formulation were determined and the results were within the specification with the accuracies obtained were within 98.1% to 101.9%. This method involves a quick analysis, cost effective and without any sample pre-treatment process. This measurement method can be very useful for determining pesticide formulations in routine analysis.

Keywords: commercial pesticide formulations, internal standard method, GC-ECD

Introduction

Pesticides are essential for the provision of food requirements for the world's growing population. The pesticide formulations used have been developed with specific pesticidal properties that are dependent upon the inherent chemical functionality and physical characteristics of constituent chemicals.

Commercial pesticide formulations contain, in addition to the active ingredients, impurities or manufacturing residual products originating from the secondary reactions in the synthesis or from reagents used in the process. The accurate, reliable analysis of all components in pesticide formulations is important to public health because of their many field applications and the non-pesticidal components can facilitate pesticide dermal absorption, the major route of exposure, as well as permeation through protective materials [1].

Different organizations, such as the Collaborative International Pesticide Analytical Communities Council (CIPAC) and the Associations of Analytical Communities International (AOAC Int.) have developed official methods for the determination of pesticides in commercial formulations. An analytical method presented for a collaborative trial through AOAC or CIPAC is a method developed by a manufacturing company [2]. Hence, these methods are valid only for particular formulations prepared by specific manufacturers. These methods are optimized for those specific products and conditions. Each chromatographic method has its own stationary phase, internal standard and mobile phase [2]. Due to the great variety of active ingredients and formulations of pesticides to be monitored, the need for new methods with higher sample output and lower costs of analysis has become imperative.

Although a great number of studies regarding the determination of active ingredient in pesticide formulation have been proposed [2-5], most of the studies only determined one or two known active ingredients from the impurities of the technical material and components of formulations at the same time. The sample output was low and the methods used are not economical.

A multi-pesticide method is one that determines the active ingredients content of each of a range of commercial pesticide formulations using the same chromatographic column and elution system [2]. In this method, many active ingredients are analyzed simultaneously. This can lead to economic reductions and increased safety without sacrificing the analytical integrity of the method. The economic savings would come in the form of fewer columns, less solvents and more efficient use of time. Safety would be increased because less laboratory waste would be generated. Furthermore, a decrease in waste would also save money because proper waste disposal is expensive.

The aim of this study is to establish and validate of a gas chromatography multi-pesticide method for the simultaneous determination of nine active ingredients (acephate, carbaryl, dimethoate,

diazinon, chlorothalonil, malathion, chlorpyrifos, quinalphos, profenofos) in their commercially available formulations which are widely used in Malaysia.

Experimental

Chemicals and reagents

All solvents used were HPLC grade. Methanol was purchased from Fisher Scientific, Loughborough, U.K. Ultra-pure distilled water and methanol were filtered through a 0.45 µm membrane filter purchased from Millipore.

Nine pesticides standards which are widely used by local farmers in fruit and vegetable cultivation namely, acephate, carbaryl, chlorpyrifos, chlorothalonil, diazinon, dimethoate, malathion, profenofos, quinalphos, were more than 95% purity and purchased from AccuStandard Inc. New Haven CT. U.S.A. 1-chloro-4-fluorobenzene (98.0%) was purchased from AccuStandard and used as the internal standard in the pesticide formulation analysis.

Materials

The nine crude samples of pesticide (pesticide formulation) were obtained from the Department of Agriculture (DOA), Ministry of Agriculture, Malaysia, and a local supplier, namely, Sin Theong Sdn. Bhd. Table 1 shows the list of generic pesticides used in this study.

Sample Preparation

The sample solution was prepared by weighing 1.00 g of the sample in a 100 mL volumetric flask and then made up to the volume with methanol. The solution was serially diluted to the concentration range of interest with methanol and a known constant amount of the internal standard was added. Then, the solution was sonicated for 10 min in an ultrasonic bath to homogenize the sample solutions before it was injected into the GC-ECD system for quantitative analysis.

Gas Chromatography – Electron Capture Detector (GC-ECD)

A Shimadzu GC 17A version 2.21 gas chromatograph with an electron capture detector was used. A SGE BPX5, 30 m x 0.32 mm i.d. capillary column with a 0.25 µm film was used in combination with the following oven temperature program: initial temperature 120 ^{o}C, then heated at 7 ^{o}C/min to a final temperature at 250 ^{o}C and held for 4.5 min. The total run time was 23.07 min. The injection volume was 2 µL with the split mode ratio of 1:36. The injector temperature was set at 250 ^{o}C and the detector temperature was set at 300 ^{o}C. Nitrogen gas (99.999%) was used as the carrier gas with a gas flow at 24.4 cm/sec linear velocity and the pressure maintained at 94 kPa.

Determination

In the determination of pesticide formulations, a series of standard mixture stock solutions for GC-ECD analysis were prepared by serially diluting with methanol until the six concentration levels were obtained. To each calibration standard, a known constant amount of internal standard (1-chloro-4-fluorobenzene, 200 µg/L) was added. The response of the peak area against concentration of standard solutions and internal standard was

Table 1 : The Generic Pesticides Used in the Pesticide Formulation Experiments.

No	Brand Name of Commercial Formulation	Active ingredient	Physical form	Labelled Value (%)	Source
1	Ortin	Acephate	Soluble powder	73.0	DOA
2	Wesco 85	Carbaryl	Soluble powder	85.0	Sin Theong
3	Lorsban	Chlorpyrifos	Emulsify concentrate	37.1	DOA
4		Chlorothalonil	Soluble concentrate	12.30	DOA
5	WA Diazinon	Diazinon	Emulsify concentrate	55.0	Sin Theong
6	Rogor	Dimethoate	Emulsify concentrate	40.0	DOA
7	Wesco 84	Malathion	Emulsify concentrate	84.0	Sin Theong
8	Selecron 500 EC	Profenofos	Emulsify concentrate	45.0	Sin Theong
9	Sandoz	Quinalphos	Emulsify concentrate	10.9	DOA

tabulated. The Response Factor (RF) for each analyte was calculated using the following equation. The RF is a unitless value:

$$RF = \frac{(A_S)\,(C_{IS})}{(A_{IS})\,(C_S)}$$

Where, A_S – Response for the analyte to be measured

A_{IS} – Response for the internal standard

C_{IS} – Concentration of internal standard

C_S – Concentration of the analyte to be measured

The average RF can be used for calculation if the RF value within the working range is constant (20% RSD or less). Alternatively, the result can be used to plot a calibration curve of response ratio (A_S/A_{IS}) vs. C_S. Then, the concentration of the active ingredient in the commercial formulation was calculated from the peak area value at a particular retention time, interpolated in a calibration graph prepared for pure standards spiked with the internal standard and using the response ratio data for each injection.

Chromatographic method validation consisting of method specificity, linearity, repeatability, precision and accuracy was undertaken in order to demonstrate the suitability of the analytical method for the determination of nine active ingredients in the pesticide formulations.

Results and Discussion

The use of gas chromatography multi-pesticide method for the simultaneous determination of nine active ingredients in pesticide formulations is presented. Method validation was carried out by determining the parameters required by CIPAC (1999) guidelines [6]. According to the above-mentioned guidelines, specificity, linearity, repeatability, precision and accuracy were established for the method validation studies.

Specificity

The ability of an analytical method to distinguish the analyte to be determined from its degradation products, metabolites or known additives was investigated [6]. For this purpose, concentrated sample extracts as well as a standard mixture of pesticides were analyzed. It was found that there was no interference since no other peaks appeared at the regions of the pesticide and the targeted internal standard. This lack of interference was also demonstrated by the application of the above-mentioned analyses to a confirmation method by using GC-MS.

Linearity of Response and Range

The linearity of response was determined by analyzing in triplicates five working solutions of different concentrations for each of the tested active ingredients. For this purpose the ratio of the peak areas of the active ingredients and that of the internal standard was plotted against their concentration ratio. After the multi-point calibration was plotted, the calibration curve values, regression coefficients and linearity ranges were determined and are shown in Table 2. In the case of pesticide formulations analysis, the results can be considered as acceptable if the regression coefficients, r^2 exceeds 0.9970. Using this criterion, the calibration shown in Table 2 was considered acceptable as the regression coefficients were greater than 0.9972.

Repeatability of Injections

The repeatability of the injection technique was tested for each active ingredient separately, using the intermediate level working standard solution. Five replicate determinations were made. In the case of pesticide formulations analysis, the repeatability is considered as acceptable if the relative standard deviation (RSD) of the peak area ratios is less than 1%, which was demonstrated in this study (Table 2).

Table 2 : Statistical Parameters of Calibration and Repeatability for Pesticide Formulations

Compound	Calibration Curve	r^2	Linearity Ranges (mg/L)	Repeatability, RSD (%) (n=5)
Acephate	y=0.5981x+0.0862	0.9980	0.016-10	0.25
Carbaryl	y=0.2552x+0.1519	0.9977	0.08-20	0.16
Dimethoate	y=4.8751x+0.1541	0.9993	0.0032-2	0.57
Diazinon	y=5.2087x+0.1418	0.9994	0.0032-2	0.98
Chlorothalonil	y=7.3235x+0.4078	0.9972	0.0032-2	0.31
Malathion	y=07553x+0.1371	0.9982	0.016-10	0.38
Chlorpyrifos	y=84.8472x+0.9388	0.9998	0.0002-0.1	0.33
Quinalphos	y=0.6011x+0.1097	0.9981	0.016-10	0.86
Profenofos	y=16.2964x+0.1718	0.9972	0.0007-0.35	0.69

Precision of the Method

Precision is the degree of agreement between independent analytical results obtained under the same analytical conditions [6]. It is a measure of random errors, and may be expressed as repeatability and reproducibility. Precision is an important characteristic in the evaluation of all quantitative methods. Repeatability and reproducibility are expressed as relative standard deviation (RSD) of a number of samples [6]. The expected repeatability and reproducibility values can be obtained from the Horwitz equation (Equation 1) and the modified Horwitz equation (Equation 2) [6]. The results are considered acceptable if they are smaller than the values calculated by the Horwitz equation.

$$RSD_R = 2^{(1-0.5 \log C)} \quad (1)$$

$$RSD_r\ (\%) = RSD_R\ (\%) \times 0.67 \quad (2)$$

Where C is the concentration of the analyte in the sample expressed as a decimal mass fraction (1 mg/L = 10^{-6}), RSD_R is the inter-laboratory relative standard deviation and RSD_r is the repeatability relative standard deviation. The data obtained from the analysis of triplicate samples were used to calculate the experimental RSD_r values. The Horwitz equation (Equation 1) and the modified Horwitz equation (Equation 2) were applied for the calculation of the expected values of RSD_R and RSD_r respectively. Table 3 shows the comparison of the experimental RSD_r values and the theoretical RSD_r values. It can be seen that the repeatability of the method is acceptable as the measured values are not outside the recommended theoretical values.

Table 3 : Results of Nine Pesticide Formulations Determination at Three Concentration Levels

Compound	Active Ingredient (%)	RSD_r (%)	Conc (mg/L)	Content in Formulation (%)	Accuracy, % (RSD_r, %)
Acephate	73	1.41	0.05	72.7	99.5 (1.0)
			0.5	73.0	100.0 (1.2)
			5.0	72.8	99.7 (1.0)
Carbaryl	85	1.37	0.1	85.8	100.9 (1.0)
			1.0	85.1	100.2 (1.0)
			10.0	84.0	99.6 (1.3)
Dimethoate	40.0	1.56	0.01	39.6	99.0 (1.0)
			0.1	39.9	99.8 (0.7)
			1.0	40.3	100.7 (0.6)
Diazinon	55.0	1.84	0.01	54.8	99.7 (1.0)
			0.1	55.0	100.1 (1.1)
			1.0	55.0	99.9 (1.2)
Chlorothalonil	12.3	1.47	0.01	12.1	98.3 (1.2)
			0.1	12.2	99.2 (1.2)
			1.0	12.3	100.1 (1.3)
Malathion	84.0	1.54	0.05	85.1	101.3 (1.1)
			0.5	84.0	100.0 (1.1)
			5.0	84.2	100.3 (1.1)
Chlorpyrifos	37.1	1.38	0.001	37.4	100.7 (1.3)
			0.01	36.4	98.1 (1.3)
			0.10	37.0	99.8 (1.2)
Quinalphos	10.9	1.51	0.05	10.8	98.8 (0.3)
			0.5	10.9	99.9 (1.5)
			5.0	10.9	100.2 (1.0)
Profenofos	45.1	1.87	0.001	44.8	99.3 (1.4)
			0.01	44.5	98.8 (1.1)
			0.10	46.0	101.9 (1.6)

Accuracy of the Method and Sample Analysis

The accuracy of a procedure may be determined by the determination of a number of samples containing a known quantity of the analyte. The mean recovery (%) for the synthetic formulation is as follows:

$$\text{Mean recovery (\%)} = \frac{\text{Mean content determined (\%)}}{\text{Theoretical content (\%)}} \times 100\%$$

Three concentration levels - at low, middle and high regions of the linear ranges were determined and the mean percentage recovery was calculated for each concentration level. Table 3 shows the results of nine pesticide formulations. The analytical results of these investigated pesticides were within the specifications for the commercial pesticide formulations.

These mean recoveries (%) should be within the following ranges:

Active ingredient, nominal (%)	Mean recovery (%)
>10	98.0 – 102.0
1 – 10	97.0 – 103.0
<1	95.0 – 105.0

(CIPAC, 1999) [6]

It should be stressed that by using the internal standard method, the error due to sample manipulation can be eliminated when taking extremely small sample volumes (2 µL). The manual injection technique was applied in this study to introduce liquid samples in the GC system. This method has significant discrimination since the uneven injection volume and injection speed will directly affect the outcome and also the precision of the results. By using the internal standard method, the error arising out of these inconsistent injections can be eliminated or minimized. The precision (RSD from 0.3% to 1.6%) obtained in this study are better than the precision reported by Skoulika *et al*. (RSD, 0.1 – 7.8%) [7], Quintas *et al*. (RSD, 1.1 – 2.6%) [8], and Kumar *et al*. (RSD, 0.87 – 2.57%) [4]. Therefore, the internal standard procedure developed in this study is suitable for the determination of the active ingredients in commercial pesticide formulations.

Conclusions

The gas chromatography multi-pesticide with an internal standard method has been successfully used for the rapid quality control of commercially available formulations of pesticides. The proposed method is a fast alternative to the FTIR procedures which is usually employed in the quality control process of commercial formulations. The main advantages of the method are that: (a) it can be performed without any sample pre-treatment. (b) it provides a high sampling throughput, because it only needs 5 min sample preparation and 20 min for the GC analysis. (c) it reduces drastically the amount of solvent used. (d) it is cost effective. This technique can be used for the quantitative determination as well as for positive identification of the active ingredients in the pesticide formulations. It can also be used to determine the percentage of active ingredients in the non-scheduled pesticides which might be used illegally.

Acknowledgements

The authors would like to acknowledge the support given by Universiti Tenaga Nasional and University of Malaya. Besides, the authors would also like to thank the Malaysia Toray Science Foundation for the financial support.

References

1. Lin, Y. W. and Hee, S. S. Q. (1998) Simultaneous gas chromatographic-mass spectrometric quantitation of the alkybenzene inert components, pesticide manufacturing by-products and active ingredient in two malathion formulations. *J. Chromatogr. A.*, **814**. 181-186.
2. Helen K., George B., Adamantia H. Abd Arpad A. (2006) Development and single-laboratory validation of a new gas chromatographic multi-pesticide method of analysis of commercial emulsifiable concentrate formulations containing alachlor, chlorpyrifos methyl, fenthion and trifluralin as active ingredients . *J. Chromatogr. A.*, **1129**. 300-303.
3. Armenta, S., Garrigues, S. and Guardia, M. D. L. (2007) Partial least squares-near infrared determination of pesticides in commercial formulations. *Vibrational Spec.*, **44**. 273-278.
4. Kumar, K. S., Suvardhan, K., Rekha, D., Kiran, K., Jayaraj, B., Janardhanam, K. and Chiranjeevi, P. (2007) Development of simple and sensitive spectrophotometric method for

the determination of bendiocarb in its formulations and environmental samples. *Environ. Monit. Assess.*, **127**. 67-72.
5. Subrahmanyam, P., Krishnapriya, B., Reddy L. R., Jayaraj, B. and Chiranjeevi, P. (2007) Spectrophotometric determination of Fenitrothion in formulations and environmental samples. *Talanta*, **72**. 106-112.
6. CIPAC, *"Guidelines on method validation to be performed in support of analytical methods for agrochemical formulations. Collaborative International Pesticides Analytical Council"*, (1999) Document No. 3807. Black Bear Press. Cambridge.
7. Skoulika, S.G., Georgious, C.A. and Polissiou, M.G. (2000) FT- Raman spectroxcopy-analytical tool for routine analysis of diazinon pesticide formulations. *Talanta*, **51**. 599-604.
8. Quintas, G., Armenta, S., Morales-Noe, A., Garrigues, S. and Guardia, M. D. L. (2003) Simultaneous determination of Folpet and Metalaxyl in pesticide formulations by flow injection Fourier transform infrared spectrometry. *Anal. Chim. Acta*, **480**. 11-21.