Simultaneous Determination of Diazinon, Malathion and Quinalphos Pesticide Formulations by Gas Chromatography with an Electron Capture Detector

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ABSTRACT A gas chromatography (GC) method has been developed to analyze simultaneously separate formulations of Diazinon, Malathion and Quinalphos with the internal standard method. A mixture of pure standard solution spiked with Chlorpyrifos as the internal standard and diluted with methanol was sonicated to homogenize the solutions. A 2µL volume of the mixture standard solution 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 the internal standard and diluted with methanol were injected into the GC-ECD. Combinations of three different concentrations of each formulated pesticide were analyzed six times to obtain coefficients of variation which are less than 1%. The total analysis time for each run was 15 minutes. The concentration of active ingredients in the pesticide formulations were calculated from the peak area values at a retention time interpolated from the calibration graphs. The analytical results which show 55.41 ± 0.82 % for Diazinon, 83.97 ± 0.36% for Malathion and 10.89 ± 0.89 % for Quinalphos were within the specifications for these commercial pesticide formulations.

ABSTRAK Suatu kaedah kromatografi gas untuk menganalisa formulasi Diazinon, Malathion dan Quinalphos secara serentak dengan menggunakan kaedah piawai dalaman telah dikenalpasti. Suatu larutan piawai tulen yang dicampur dengan chlorpyrifos sebagai piawai dalaman telah dicairkan dengan metanol dan disonikkan supaya menjadi larutan homogen. Isipadu larutan piawai 2µl telah disuntikkan dalam GC-ECD dan suatu keluk kalibrasi enam titik yang menunjukkan julat linear telah dihasilkan bagi setiap sebatian sasaran. Formulasi bagi setiap sampel yang bercampur dengan piawai dalaman dan dicairkan dengan metanol telah disuntik dalam GC-ECD. Tiga kombinasi formulasi dengan kepekatan yang berbeza telah dianalisa sebanyak 6 kali supaya mendapat koefisen variasi yang kurang daripada 1%. Jumlah masa yang diambil untuk setiap penganalisaan adalah selama 15 minit. Kepekatan ingredien aktif dalam formulasi racun haiwan perosak dikira berdasarkan nilai keluasan puncak pada masa retensi daripada keluk kalibrasi. Keputusan analisis menunjukkan peratusan sebatian sasaran Diazinon 55.41 ± 0.82%, Malathion 83.97 ± 0.36% dan Quinalphos 10.89 ± 0.89%. Nilai-nilai ini adalah dalam lingkungan spesifikasi bagi ketiga-tiga formulasi racun perosak komersil.

(GC-ECD, pesticide formulation, Diazinon, Malathion, Quinalphos)

INTRODUCTION

Pesticides are agrochemicals designed to combat the attack of various pests on agricultural and horticultural crops. Pesticides in fruits and vegetables can be a significant route to human exposure [1, 2]. Pesticides are applied to fruits and vegetables at various stages of cultivation and during post-harvest storage to provide protection against a range of pests, before they become available to consumers. The formulations used have been developed with specific pesticidal properties that are dependent upon the inherent

chemical functionality and physical characteristics of constituent chemicals.

The use of agrochemical formulations with more than one active principle is a common practice in order to improve the crop protective action. The determination of every active compound in this type of product requires in many cases the use of a variety of analytical techniques. Statutory maximum residue levels for pesticides in foodstuffs have been defined in most countries to guarantee consumer safety and to regulate international trade [3, 4]. The determination of pesticide residues in foodstuffs is then a requirement to support enforcement of legislation, to ensure trading compliance, and in the conduct of surveillance programmes to monitor residues in regional and national dietary components [5].

Diazinon, malathion and quinalphos are compounds with heterogeneous structures, used for insects, mites and diseases control in fruits and vegetables. Their residues can be present simultaneously in these samples constituting an important risk for human health owing to their chronic toxicity [6].

Diazinon (o,o – diethyl o-2-isoproyl-6-methyl pyrimidine-4-yl) phosphorothiate is a nonsystemic organophosphate insecticide used to control cockroaches, silverfish, ants and fleas in residential and non-food buildings [7]. It is used on home gardens and farms to control a wide variety of sucking and leaf eating insects. It is also used on rice, fruit trees, sugarcane, corn, tobacco, and potatoes and on horticultural plants. Diazinon has veterinary uses against fleas and ticks. It is available in dust, granules, seed dressings, wet table powder and emulsifiable solution formulations. Toxic effects of diazinon are due to the inhibition of acetyl cholinesterase, an enzyme needed for proper nervous system function. The range of doses that results in toxic effects varies widely with formulation and with the individual species being exposed. The toxicity of encapsulated formulations is relatively low because diazinon is not released readily while in the digestive tract. Some formulations of the compound can be degraded to more toxic forms. This transformation may occur in air, particularly in the presence of moisture and by ultraviolet radiation. However, most modern diazinon formulations are stable and do not

degrade easily [8]. In fact, a low temperature and high oil content tend to increase the persistence of diazinon [9]. Generally the half life is rapid in leafy vegetable, forage crops and grass. The range is from 2 to 14 days. Diazinon is absorbed by plant roots when applied to the soil and translocated to other parts of the plant. Preparations used in agriculture contain 50 - 90% diazinon and appear as a pale to dark brown liquid [10].

Quinalphos (o,o-diethyl o-quinoxalin-2 phosphorothiate) is an organophosphorus pesticide used in the control of pests on fruit vegetables, cereal, rice, wheat, maize and cotton. Pure quinalphos is a colourless crystalline solid and the technical material is a liquid. It is applied as an emulsifiable concentrate, granules, wettable powder, liquid or dispersible powder. Apart from its insecticidal properties, quinalphos is known to induce various toxic effects in nontarget species. The oral LD50 values range from 14 to 137 mg/kg in rats. In studies of human poisoned by quinalphos, the cholinesterase activity was affected not only by the amount of pesticides absorbed but also by retention in the body and excretion of metabolite [11]. Its use and behavior have been thoroughly investigated [12 - 15].

Malathion [diethyl (dimethoxythiophosphorylthio) succinate, is a non systemic, wide spectrum organophosphorus insecticide. It is employed for the control of insects on fruits and vegetables and is also used to control mosquitoes, flies, household insects and animal parasites, head and body lice. It is available as an emulsifiable concentrate, wettable powder, dust able powders and ultra low volume liquid formulations. Malathion is slightly toxic via oral and dermal routes. The effects of malathion are similar to those observed with other organophosphates, except that larger doses are required to produce the effects [16]. Animal tests on malathion formulations indicate that it can affect the central nervous system, immune system, adrenal gland, liver and blood. Malathion has a low persistence in soil with half-lives of 1-25 days. Breakdown occurs by a combination of biological degradation and non-biological reaction with water. If released to the atmosphere, malathion will break down rapidly in sunlight, with reported half-life in air of about 1.5 days [17].
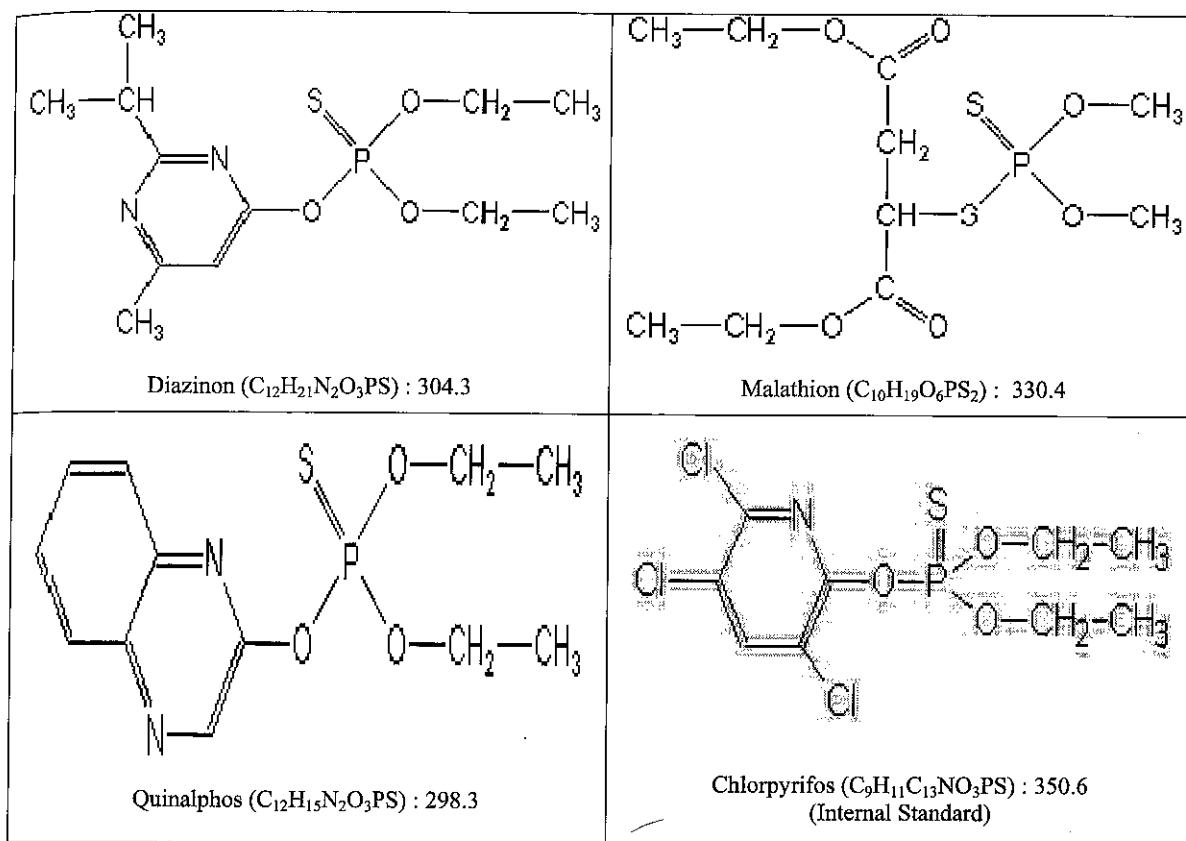
Figure 1. Pesticides Studied and Their Molecular Weight

The recommended Collaborative International Pesticides Analytical Council (CIPAC) method for the determination of diazinon, quinalphos and malathion in pesticide formulations are based on gas chromatography with flame ionization detection (GC-FID) [12]. Other procedures proposed for determination of pesticide formulations of diazinon, quinalphos and malathion are GC with mass spectrometry detection [18], GC with nitrogen-phosphorous detection and GC – tandem mass spectrometry [19].

Similarities in their chemical structures and properties enable diazinon, quinalphos and malathion to be 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 a rapid and accurate method to determine and quantify the active ingredients in commercial diazinon, quinalphos and malathion formulations simultaneously using gas chromatography with an electron capture detector.

MATERIALS AND METHODS

Reagents and Solutions
a. Solvent: methanol (HPLC grade) was obtained from Fischer Scientific, Loughborough, UK and is filtered through a 0.45 µm filter from Millipore.
b. Pesticide standards: diazinon (98.9%), quinalphos (100%) and malathion (96.4%) were purchased from Accu Standard Inc, New Haven CT, USA. Stock mixture solutions of diazinon, quinalphos and malathion of 100 ppm were serially diluted in methanol ranging from 0.5 - 20ppm.
c. Commercial formulation: quinalphos (10.9%) was obtained from the Pesticide Control Division, Department of Agriculture. diazinon (55.0%) and malathion (84.0%)

were purchased from Sin Teong Hing Sdn. Bhd.

d. Internal Standard: chlorpyrifos (100%) in methanol solution (100 µg/mL) was purchased from Accu Standard.

Instruments

A Shimadzu GC-17A fitted with an Electron Capture Detector (ECD) – ECD 17 ver. 2 was used. Nitrogen (99.999 % purity) was used as the carrier gas in the GC. Analyses were carried out with a SGE 30m QC2/BPX5 capillary column where BPX5 is 5% phenyl equivalent modified siloxane of 30 m long x 0.32 mm ID. The integrator used is a Shimadzu C-R6A Chromatopac. Isothermal condition was applied in this study. Nitrogen carrier gas flow was established at 32 cm/sec linear velocity and the pressure was at 69 kPa. The oven temperature was 230 °C. The injection volume was 2 µL with the split mode ratio of 1:36. The injector temperature was 250 °C and the detector temperature was 300 °C.

Analytical Procedures

For the internal standard calibration, a series of standard diazinon solutions containing 6 concentration levels were prepared by serially diluting with methanol. To each calibration standard, a known constant amount of internal standard (0.5 ppm chlorpyrifos) was added. For the analysis of the active ingredient in the commercial formulation, 1.00 g of diazinon commercial pesticide formulation was accurately weighted into a 10 mL volumetric flask and dissolved with methanol. The solutions were serially diluted with methanol and a known constant amount of internal standard was added. The solutions were sonicated for 5 minutes in an ultrasonic water bath to homogenize the sample solutions [12]. 2 µL was injected into a gas chromatograph fitted with an ECD. This procedure was repeated for the determination of malathion and quinalphos.

Determination

First, the retention time of each individual compound should be confirmed by using a standard solution and a mixture of standards solution. After that, comparing the retention times of the peaks with the standard chromatogram under the same temperature and

same instrumental conditions would yield identification of separated species.

For quantitative analysis, the response of the peak area against concentration of standard solutions and internal standard was tabulated. The Response Factor (RF) for each analyte was calculated using the following equation. The RF is a unit-less value [20].

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

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

If the RF value within the working range is constant (20% RSD or less) the average RF can be used for the calculation. Alternatively, the result can be used to plot a calibration curve of response ratio (A_S/A_{IS}) vs. C_S [20]. Then the concentration of the active ingredient in the commercial formulations was calculated from peak area value at a 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. The repeatability of each peak area measurement was evaluated for 10 consecutive injections. 8 out of 10 results were used for the calculation.

RESULTS AND DISCUSSION

Diazinon, quinalphos and malathion are not formulated together but, because of the similarities in their chemical and physical properties, a procedure could be developed to extract and measure their formulations simultaneously. Chromatographic methods based on GC can be used for quantitative determination of active ingredients in pesticide formulation. Our studies on gas chromatography were focused on gas chromatography with an electron capture detector since it is widely used in quality control laboratories of agrochemicals and also because it is sensitive to our compound of interest.
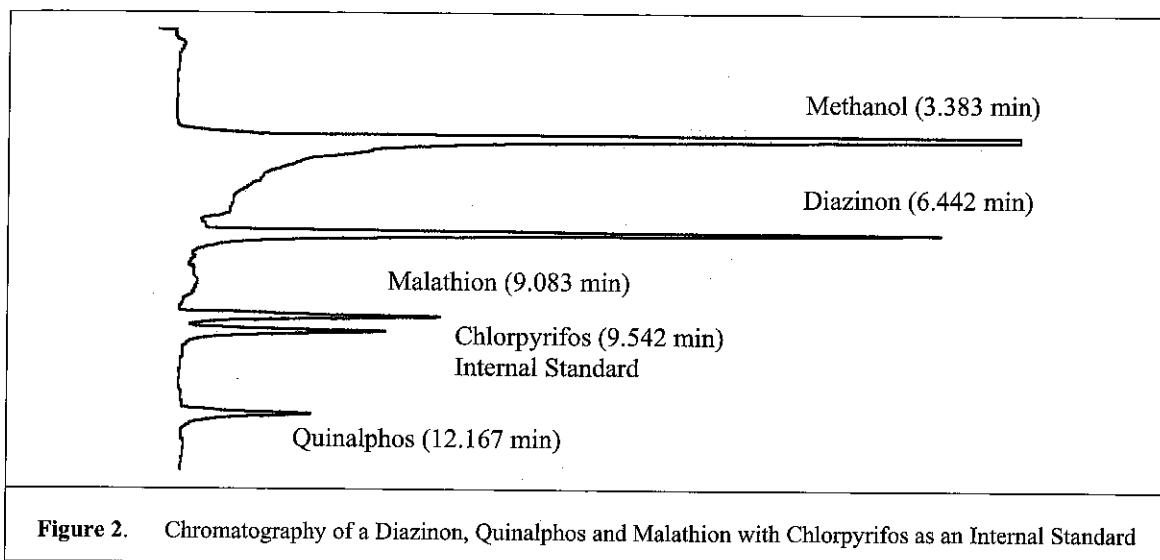
Figure 2. Chromatography of a Diazinon, Quinalphos and Malathion with Chlorpyrifos as an Internal Standard

A typical chromatogram of a Diazinon, Quinalphos and Malathion with Chlorpyrifos as an internal standard is shown in Figure 2. Baseline resolution of the 3 compounds and the internal standard is completed in 12.20 min.

Using the GC-ECD system, calibration graphs of each pesticide were prepared by injecting 6 mixtures standard solutions of diazinon, quinalphos and malathion, which were prepared by appropriate dilution of the respective stock solution. A series of six point calibration curves were constructed using the ratio of peak area of investigated pesticides against internal standard and concentration of the respective pesticide standard solutions. The calibration curve of each investigated pesticide is shown in Figure 3.

To demonstrate the ruggedness of the method, three different concentrations of formulations for each investigated pesticide were analyzed 10 times. 8 out of 10 results were used for the calculation. Furthermore, the linearity of response for all investigated compounds was checked and the responses were linear for the ranges of 10 ppm, 15 ppm and 20 ppm for diazinon and malathion. The linearity of response quinalphos is from 50 ppm, 80 ppm and

100 ppm. These linearity ranges are acceptable for formulations that are in the low or high concentration levels. The sensitivity of this method was excellent for all compounds. The relative standard deviation (RSD) obtained were below 1 %. The internal standard method minimizes the error due to manual injection. Table 1 presents the statistical parameters of the calibration curves and the result of diazinon, quinalphos and malathion analysis. The analytical results of the three investigated pesticides were within specifications for the commercial pesticide formulations.

It should be stressed that by using the internal standard method, the error due to sample manipulation was eliminated and especially when taking extremely small sample volumes (2µL). The manual injection technique was applied in this study to introduce liquid samples into 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 problem of these inconsistent injection can be eliminated or minimized.
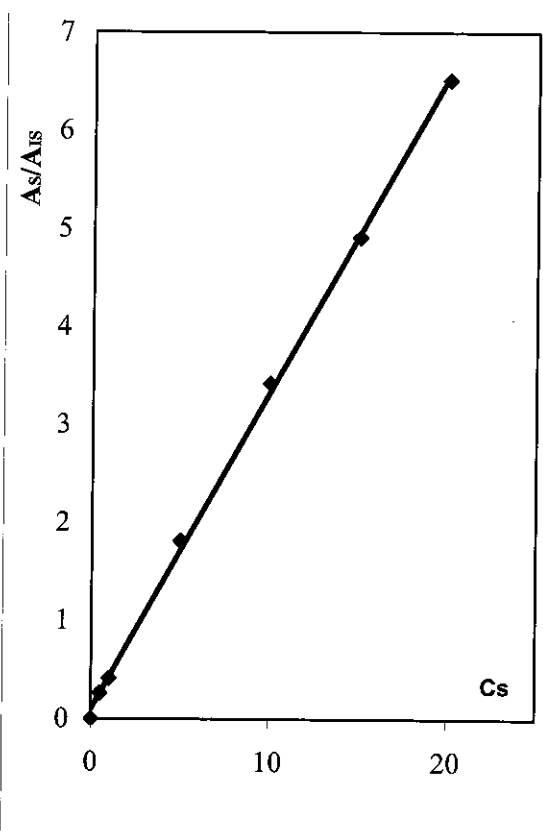
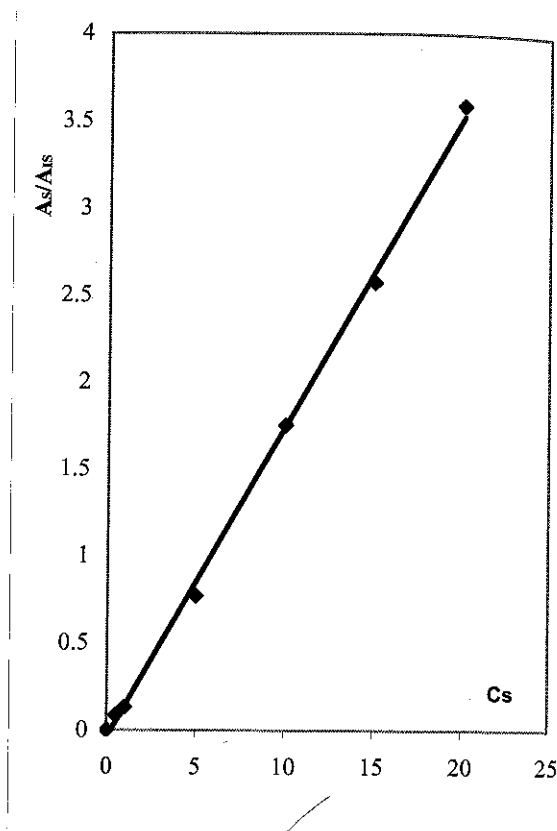**Figure 3a.** Calibration Curve of Diazinon
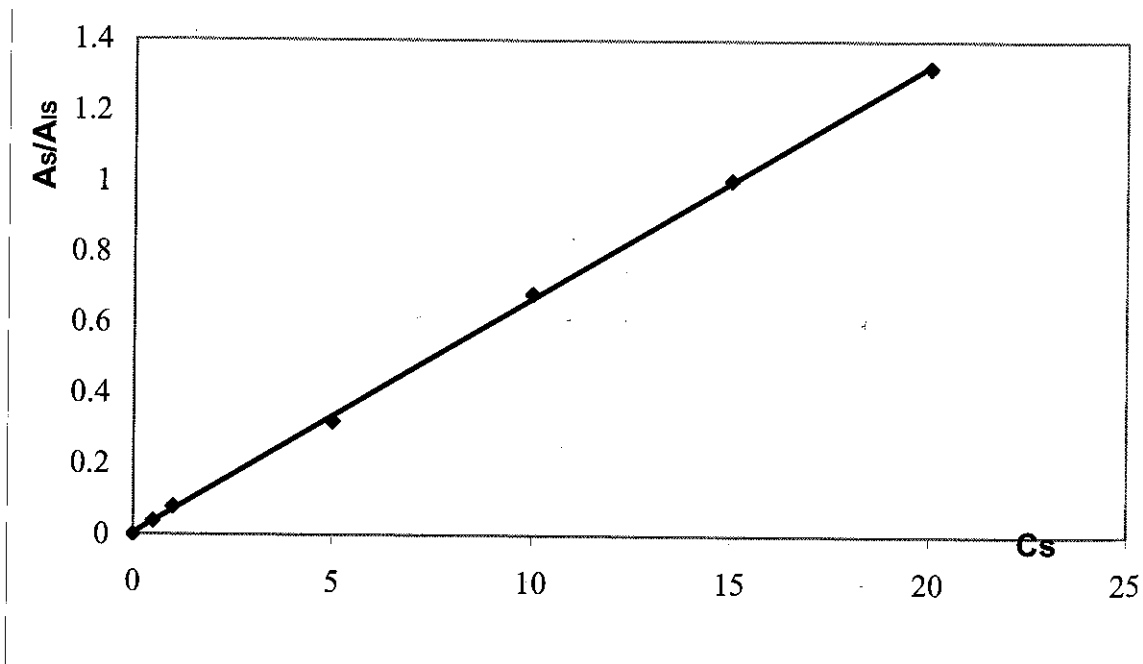

Figure 3b. Calibration Curve of Malathion

Figure 3c. Calibration Curve of Quinalphos

Table 1.   Statistical Parameters of Calibration and Results of Formulation

COMPOUND	DIAZINON	MALATHION	QUINALPHOS
Calibration Curve	Y = 0.3232x + 0.0997	Y = 0.1786x-0.041	Y = 0.0663x+0.0046
Corre. Coefficients (R^2)	0.9992	0.9985	0.9996
Mean content in formulation (x ± RSD %)	55.41 ± 0.82 %	83.97 ± 0.36	10.89 ± 0.89 %
Actual formulated amount	55.00 %	84.00	10.90 %

CONCLUSION

The combined analysis of active ingredients in commercial diazinon, quinalphos and malathion pesticide formulation using this method is simple, rapid, specific, reproducible and can be carried out without any pre treatment of samples. Furthermore, this method contributes to cost savings and increased safety. Besides, it can solve the problems which are caused by manual injection.   The proposed procedure is an environmentally friendly alternative for quality control analysis for formulated pesticides.

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