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Validation of HPLC Method for Quantitative Determination of Pirimiphos methyl

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ABSTRACT

The objective of this research to optimise the HPLC method was developed for quantitative determination of Pirimiphos-methyl. Chromatographic separation was achieved on a 250 x 4.6 mm i.d. reversed phase column Qualisil BDS 5u C18, Using deionized acetonitrile:water in the ratio of 85:15 v/v respectively as mobile phase. The eluent was monitored at 254 nm. A sharp peak was obtained for the Pirimiphos-methyl at 9.29 min. The UV Spectrophotometric method performed at 254 nm after full scan analysis using methanol as a solvent. The result revealed that both methods are suitable to carry out routine analysis of Pirimiphos methyl, However HPLC results showed high precise, accurate and sensitive than the UV Spectrophotometer. Hence HPLC method is suitable for trace analysis of Pirimiphos methyl in environmental samples.

***Keywords*:** Pirimiphos methyl; HPLC; UV-spectrophotometric; Linearity; Precision; Recovery; Quantification; LOD and LOQ

1. INTRODUCTION

Pirimiphos methyl [O-2-(diethylamino)-6-methylpyrimidin-4-yl O,O-dimethyl phosphorothioate] is an phosphorothioate, which is a broad-spectrum insecticide and acaricide with contact and respiratory action. It shows activity against a wide variety of insects including ants, aphids, beetles, caterpillars, cockroaches, fleas, flies, mites, mosquitoes, moths, and thrips. It penetrates the leaf tissue and exhibits translaminar action. It control of a wide range of insects and mites in warehouses, stored grain, animal houses, domestic and industrial premises. Pirimiphos methyl is rapidly absorbed, metabolized and excreted in rats and dogs. In both species, 2-ethyl amino-4-hydroxy-6-methyl pyrimidine is the major metabolite [1]. The reproductive organs have been shown to be among the most vulnerable organs to organophosphorous insecticides [2-4].

Pirimiphos-methyl is a cheap pesticide widely used in the world and particularly in Africa to protect food against pests. Pirimiphos-methyl is a fast-acting broad spectrum insecticide with both contact and fumigant action. Pirimiphos-methyl has a half-life [5] of 117 days in water (Yamada 2005; Bullock 1974), 180-270 days on greens and seeds, and 130 days in soil (Bowker et al. 1972). The chemical name of pirimiphos methyl [6] is *O*-(2-diethylamino-6-methylpyrimidin-4-yl)-*O,O*-dimethyl phosphorothioate. Its structural formula can be seen in Figure 1.

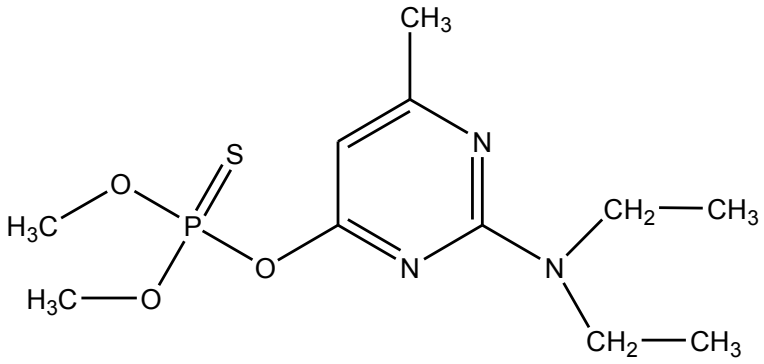


Figure 1. Chemical structural of pirimiphos methyl.

Different chromatographic methods followed for the determination of Pirimiphos methyl in food and environmental samples [7-8]. For example, limits of detection (LOD) between 0.05 and 0.5 ng/ml were reached using thin layer chromatography (TLC) [9]; also high performance liquid chromatography (HPLC) with LOD of 16 ng/ml for the analysis of Pirimiphos methyl in water [10-11]. In addition, gas chromatography (GC) coupled to nitrogen/phosphorous (NPD) [12-13], atomic emission (AED) [14], electron capture (ECD) [15] or flame photometric detectors (FPD) [16] have also been applied for the determination of Pirimiphos methyl. The aim of this paper is to develop and validate HPLC and UV visible method using a suitable conditions method for the determination of Pirimiphos methyl.

2. MATERIALS AND METHODS

Solvents (acetonitrile, methanol and reference standard Pirimiphos methyl) and HPLC water used for this study were sigma aldrich and merck respectively.

2. 1. Instrumentation and analytical conditions

HPLC analyses were performed using the LC-10AT VP and SPD-10A UV-VIS Detector of Shimadzu with PC integrator. A reversed-phase column (Qualisil BDS 5u C18, Size-250 x 4.6 mm (i.d); particle size 5 μm). The room temperature maintained at 32 °C. The mobile phase was a mixture of acetonitrile /water (85/15, v/v) with a flow rate of 1.0 mL/min. the detection was at 254 nm when the injection volume was 20μL.

The UV spectrophotometric method was performed using shimadzu UV-Visible double beam spectrophotometer model 1700 pharma spec. UV-Visible is controlled by PC and UV probe personal software package. The analysis was performed with methanol as a solvent.

2. 2. Linearity

Preparation of Stock solution:

Accurately weighed 137.0 mg of Pirimiphos-methyl standard (99.50 % purity) in 100 ml standard flask, added sufficient volume of mobile phase to dissolve the contents and make up the volume up to the mark of standard flask.

Preparation of calibration solutions:

From the above stock solution, made serial dilution for further preparation of concentrations such as 9.59, 10.96, 12.33, 13.70, 15.07 and 16.44 mg in 100 ml standard flasks separately. The standard solutions were allowed to stand for 30 minutes at room temperature for equilibration.

Injected each standard in the HPLC system thrice and recorded the corresponding peak areas were presented in Table 1 and calibration chart were presented in Figure 2.

Table 1

Weight (mg)	Area (mV·Sec)	Mean Area ± SD (mV·Sec)
9.59	9182.6374	9182.5869 ±0.4943
	9183.0541	
	9182.0693	
10.96	10642.3293	10643.2536 ±1.3040
	10644.7452	
	10642.6863	
12.33	11981.0531	11980.3312 ±0.9501
	11979.2549	
	11980.6857	
13.70	13306.9827	13306.9894 ±0.3342
	13307.3269	
	13306.6587	
15.07	14638.3305	14637.9342 ±0.9272
	14638.5974	
	14636.8748	
16.44	16329.3297	16330.3468 ±1.4200
	16331.9692	
	16329.7416	

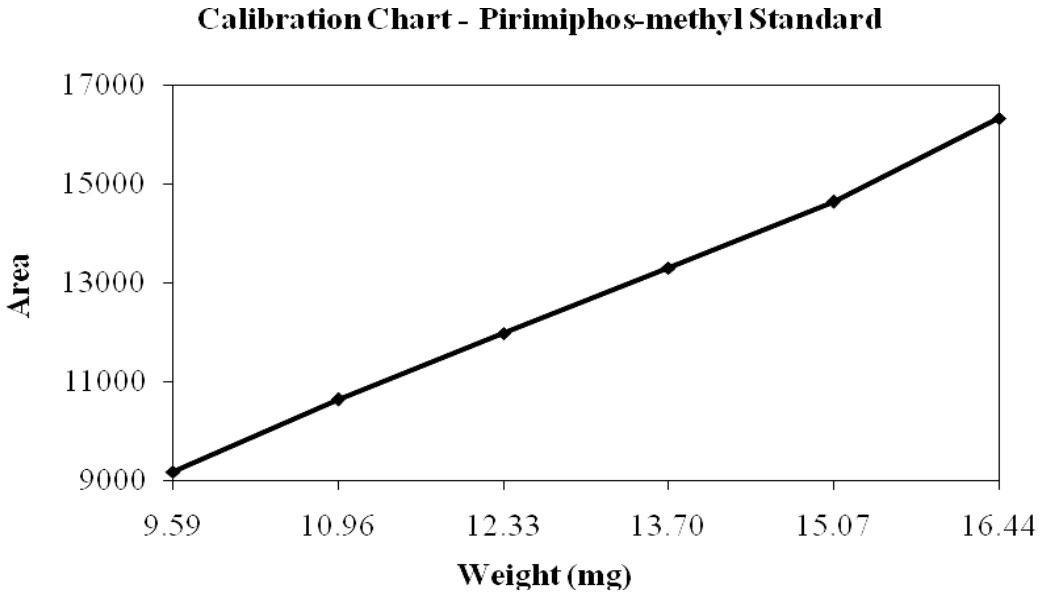


Figure 2. Calibration chart of Pirimiphos-methyl.

Statistical Parameters

Slope	1.0482
Correlation	0.9992
Intercept	2.9346

2. 3. Precision

Preparation of Standard Solution:

Use the concentration of 4th Linearity standard (13.70 mg/100 ml) or Pipette out 10 ml of stock solution into 100 ml standard flask, added sufficient volume of mobile phase up to the mark of standard flask. The solution was allowed to stand for 30 minutes in room temperature for equilibration.

Preparation of Sample solution:

Accurately weighed 14.10 mg of Pirimiphos-methyl technical sample in separate 100 ml standard flask, added sufficient volume of mobile phase to dissolve the content and make up to the mark of standard flask. The solution was allowed to stand for 30 minutes in room temperature for equilibration.

The standard and sample solutions were injected in the HPLC system continuously and recorded the respective peak areas were presented in Table 2. The Pirimiphos-methyl content was calculated using the following formula-1,

$$\text{Pirimiphos-methyl content (\%)} = \frac{A \times B}{C \times D} \times P \longrightarrow 1$$

where,

A - Area of Pirimiphos-methyl peak in sample
B - Weight of standard
C - Area of Pirimiphos-methyl peak in standard
D - Weight of sample
P - Purity of reference standard

Table 2

Sample	Weight (mg)	Area (mV·Sec)	Pirimiphos-methyl content (%)
Standard	13.70	13306.9827	-
Sample	14.10	12996.0369	94.418
		13001.2574	94.456
		12991.3941	94.385
		12998.6024	94.437
		13003.2319	94.471

Statistical Parameters

Mean - 94.433
Standard Deviation - 0.034
Relative Standard Deviation - 0.036

2. 4. Recovery

Preparation of Standard Solution:

Use the concentration of 4th Linearity standard (13.70 mg/100 ml) or Pipette out 10 ml of stock solution into 100 ml standard flask, added sufficient volume of mobile phase up to the mark of standard flask. The solution was allowed to stand for 30 minutes in room temperature for equilibration.

Preparation of Blank Sample solution:

Accurately weighed 14.0 mg of Pirimiphos-methyl technical sample in separate 100 ml standard flasks, added sufficient volume of mobile phase to dissolve the contents and make up to the mark of standard flask. The solution was allowed to stand for 30 minutes in room temperature for equilibration.

Preparation of fortified standard solutions:

Pirimiphos-methyl technical sample of 14.0 mg were taken in to three separate 100 ml standard flasks and added sufficient volume of mobile phase to dissolve the contents. To this fortified three levels of standards 0.96, 1.92 & 2.88 mg respectively. Made the standard flasks up to the mark-using Mobile phase and mixed well.

The standard and sample solutions were injected in the HPLC system continuously and recorded the respective peak areas were presented in Table 3. Then calculate the recovery using the following formula,

$$\text{Recovery (\%)} = \frac{\text{Weight recovered (mg)}}{\text{Weight fortified (mg)}} \times 100$$

Calculation:

Standard weight (mg) = 13.70
Area of standard (mV·Sec) = 13306.9827
Area of blank sample (mV·Sec) = 12955.3297

Table 3

Fortified Standard level (mg)	Fortified Sample Area (mV·Sec)	Recovery (%)
0.96	13876.6594	98.81
	13872.9990	98.41
1.92	14790.3845	98.40
	14792.4844	98.51
2.88	15714.7282	98.64
	15720.1822	98.84

Statistical Parameters

Mean - 98.60
Standard Deviation - 0.194
Relative Standard Deviation - 0.196

2. 5. Quantification

Preparation of Standard Solution:

Use the concentration of 4th Linearity standard (13.70 mg/100 ml) or Pipette out 10 ml of stock solution into 100 ml standard flask, added sufficient volume of mobile phase up to the mark of standard flask. The solution was allowed to stand for 30 minutes in room temperature for equilibration.

Preparation of Sample solution:

Weighed exactly known weight of Pirimiphos-methyl Technical sample in a 100 ml standard flask, added sufficient volume of mobile phase to dissolve the contents and make up to the mark of standard flask. The solution was allowed to stand for 30 minutes in room temperature for equilibration.

The standard & sample solutions were injected into the HPLC system. From the peak area of consecutive injection of standard solution and sample solution were presented in Table 4. The Pirimiphos-methyl content calculated as per formula-1.

Table 4

Sample weight (mg)	Sample Peak Area (mV·Sec)	Mean (mV·Sec)	Standard weight (mg)	Standard Peak Area (mV·Sec)	Mean (mV·Sec)	Content (%)
14.24	13116.0561	13116.1175	13.70	13309.2591	13309.2995	94.337
	13115.8419					
	13116.4544			13309.0548		
14.01	12893.3147	12893.5114				94.259
	12892.8526			13309.5847		
	12894.3669					

2. 6. LOD and LOQ

Limit of Detection & Limit of Quantification:

The LOD & LOQ are determined by analyzing with known diluted standard of Pirimiphos-methyl of different concentrations. From the response of the analyte concentration the signal noise ratio (S/N) was also calculated for LOD & LOQ determination were presented in Table 5.

LOD & LOQ of primiphos-methyl al content

Table 5

Conc. (ppm)	Response (Peak area)	Mean Response (Peak area)	Mean Noise Area for Blank	Signal to Noise Ratio	Remark	% of LOD & LOQ
0.01	0.9689	0.9802	0.3248	3.02	LOD	0.000001
	0.9915					
0.02	1.8493	1.8879		5.81	LOQ	0.000002
	1.9264					
0.04	3.9849	3.9392		12.13	-	-
	3.8934					

Note: LOD = signal to noise ratio 3 ±0.5; LOQ = signal noise ratio between 5 to 10

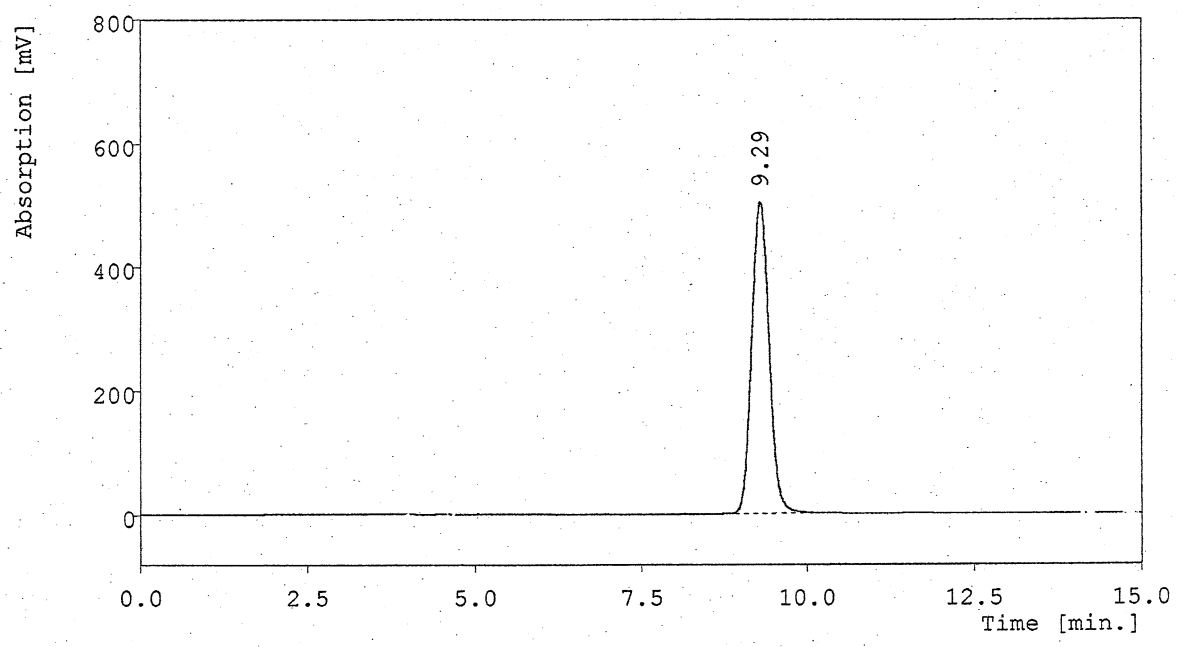
3. RESULTS AND DISCUSSION

3. 1. HPLC Method

A typical sharp, single peak of pirimiphos methyl chromatogram obtained at 9.29 min after injection with set off parameters adopted in this study. Figure 3 shows the ideal chromatogram of Pirimiphos-methyl.

The developed method was validated in terms of linearity, precision, recovery, quantification, LOD and LOQ. The standard calibration curve showed in Fig. 2 was linear over the concentration range 9.59 to 16.44 mg with a correlation coefficient (r^2) 0.9992. LOD and LOQ were found to be 0.000001 and 0.000002 % respectively, indicating the sensitivity of the method. Experiments demonstrated satisfactory accuracy with small relative standard deviations 0.36 (RSD %). The developed HPLC method was precise since the % RSD values were 0.1< for both repeatability and intermediate precision studies. RSD of repeatability (intra-day) and intermediate precision (inter-day) ranged from 0.0241 to 0.03608 and 0.0423 to 0.0662 respectively. Chromatographic conditions were carefully optimized to get satisfactory resolution of the test substance.

The final decision on mobile phase composition and flow rate was made on the basis of peak shape (peak area, asymmetry, tailing factor), baseline drift, time required for analysis, and cost of solvents. The optimized mobile phase was a mixture of acetonitrile/water/ (85/15, v/v) with a flow rate of 1.0 mL/min. the detection was made at 254 nm by making preliminary experiment on the Pirimiphos methyl with using mobile phase compostion as solvent on UV-Visible spectrophotometric showed that there was peak at 254 nm by applying the wavelength of the HPLC method showed good response with it affected on the LOD to be 0.000001 % and LOQ to be 0.000002 % ppm.



Peak #	Ret. Time	Area	Height	Area %	Name
1	9.29	9182.6374	503.4921	100.0000	Pirimiphos-methyl
		9182.6374	503.4921	100.0000	

Figure 3. A typical chromatogram of Pirimiphos methyl.

3. 2. UV-Visible Spectrophotometric method

The test substance solutions were scanned in the wavelength range 200-1100 nm after making suitable dilutions from the stock solutions. Pirimiphos-methyl showed absorption maxima at 254 and a weaker band at 300 nm. Pirimiph + os-methyl in methanol as a solvent showed linear relationship in the concentration range of 1.246 to 10 mg with a correlation coefficient (r^2) 0.9991. LOD and LOQ were found to be 1.682 and 5.1 mg respectively. The percentage of RSD value for intra-day and inter-day precision varied from 0.0453 to 0.062 and 0.0542 to 0.9255 respectively. The spectrophotometric method allowed rapid quantification of Pirimiphos-methyl. Methanol was chosen as the solvent because of good solubility.

The use of UV-visible spectroscopy as a primary method of determination in organo phosphorous pesticide quantification experiment has declined to an insignificant level in recent years. This method is based upon the measurement of the absorbency of organo phosphorous spectrophotometric reagent which is sensitive to low microgram amounts. The direct utility of a UV-visible spectroscopic method in pesticides analysis is limited because of its relatively low sensitivity and selectivity. Pirimiphos-methyl showed absorption maxima at 254 nm which was selected as the detection wavelength since it showed better linearity and sensitivity at this wavelength.

4. CONCLUSIONS

The validated HPLC and UV-Visible methods were found to be accurate, precise and reliable. UV spectrophotometric and HPLC methods are quite simpler. However instrument HPLC is not available UV spectrophotometric method can follow for routine quantification purpose. The test substance Pirimiphos-methyl quantification is very simple and rapid analysis can be performed using HPLC method without any difficulties. The validation results (linear, precise, recovery, quantification, LOD and LOQ) are confirmed the high accuracy of the method.

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