

# Validation of UV Spectrophotometric and HPLC Methods for Quantitative determination of chlorpyrifos

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## ABSTRACT

A specific and sensitive HPLC and UV spectrophotometric methods were developed for determination and analysis of chlorpyrifos. Chromatographic separation was achieved on a 150 mm x 10 mm I.D. reversed phase column Zorbax SB C-18. using deionized water: acetonitrile in the ratio of 10:90 v/v respectively as mobile phase. The effluent was monitored at 290 and 230 nm. Two sharp peaks were obtained for the solvent and chlorpyrifos at 2.7 and 3.45 min respectively. UV spectrophotometric method was performed at 290 nm using Isopropanol as the solvent. Linear range was 0.025-3500 ppm ( $r^2 = 0.9986 \pm 0.0009$ ) for HPLC method and 2.229 to 200 ppm ( $r^2 = 0.9988$ ) for UV spectrophotometric method. Validation guidelines and statistical analysis showed that both the methods were precise, accurate, sensitive, and can be used for the routine quality control of chlorpyrifos in waste discharges

**Keywords:** chlorpyrifos; HPLC; UV-spectrophotometric; liner range

## 1. INTRODUCTION

Chlorpyrifos [O,O-diethyl-O-3,5,6-trichloro-2-pyridylphosphorothioate] is an organophosphorus pesticide (OPP), which is a broadly active insecticide effective by ingestion and contact. The metabolites of chlorpyrifos being chlorpyrifos oxon, 3,5,6-trichloro-2-pyridinol and diethyl phosphorothioic acid and/or diethyl phosphoric acid [1,2]. Chlorpyrifos is stable in air (nonvolatile) and it is not sensitive to ultra violet radiation. It is stable to neutral and weakly acidic solution, but it is hydrolysed by strong bases. The rate of chlorpyrifos hydrolysis increases with both pH and temperature [3]. Chlorpyrifos acts by phosphorylating acetylcholinesterase both at the synapse of neurons and in the plasma. The inactivation of the acetylcholinesterase causes an accumulation of acetylcholine (ACh) which is normally hydrolysed by the enzyme, paralysing the synaptic site.

The manifestations of organophosphate poisoning are associated with the increase in concentration of ACh at the neuron synapse [4]. Reduction in acetylcholinesterase activity to  $10 \pm 20$  % of normal activity in man results usually in death [5]. Different chromatographic methods for the determination of chlorpyrifos in food and environmental samples [6-7]. For example, limits of detection (LOD) between 0.05 and 0.5 ng/ml were reached using thin layer chromatography (TLC) [8]; also high performance liquid chromatography (HPLC) with LOD

of 16 ng/l for the analysis of chlorpyrifos in water [9]. In addition, gas chromatography (GC) coupled to nitrogen/phosphorous (NPD) [10-11], atomic emission (AED) [13], electron capture (ECD) [13] or flame photometric detectors (FPD) [14], have also been applied for the determination of chlorpyrifos.

The aim of this paper is to develop and validate HPLC method using a suitable conditions and UV spectrophotometric method for the determination of chlorpyrifos.

## 2 MATERIALS AND METHODS

Analytically pure chlorpyrifos, (assigned purity > 97 % purity) was purchased from sigma Aldrich. Chloroform, Acetonitrile Water analytical grade -HPLC was purchased also from Sigma Aldrich.

### 2. 1. Instrumentation and analytical conditions

Analytically pure chlorpyrifos (purity > 97 %purity) were obtained from Sigma aldrich. HPLC grade chloroform, HPLC water, HPLC aceyonitril was obtained from Sigma aldrich. Isopropanol for standard solution preparation for UV-Visible.

### 2. 2. Instrumentation and analytical conditions

HPLC analyses were performed using the Agilent 1100 system equipped with an auto injector and ultraviolet detector (Agilent, Palo Alto, USA). A reversed-phase column (Eclipse XDB-C18; size: 250 mm × 4.6 mm (i.d); particle size 5 µm) from Agilent Company was used for separation and maintained at 40 °C.

The mobile phase was a mixture of acetonitrile /water/ glacial acetic (90/10/0.1, v/v/v) with a flow rate of 1.0 mL/ min.the detection was at 290,230 nm when the injection volume was 1 µL.

The UV spectrophotometric method was performed using Shimadzu UV-Vis double beam spectrophotometer model 1700.UV-Vis double beam spectrophotometer is controlled by its control panel or using PC by UV probe personal software package. UV probe is modular approach to data collection, analysis, and reporting provides a rare simplicity and power.the samples wrer prepared using Isopropanol.

### 2. 3. Preparation of standard solutions

For HPLC method, 3000 ppm of chlorpyrifos was prepared by dissolving 3 mg each in 100 ml chloroform. Working standard solutions of chlorpyrifos (1 - 30 µg/ mL) were prepared by transferring 0.1 to 3 ml of chlorpyrifos stock solution to serially arranged 10 ml standard flasks and diluting to volume using chloroform. For the UV spectrophotometric method, stock solution of 500 ppm of chlorpyrifos was prepared in isopropanol.

The working standard solutions were prepared by dilution of the stock solution with isopropanol to get a concentration range 1-500 ppm The absorbance was measured at 290 nm using isopropanol as blank.

### 2. 4. Linearity and range

For the HPLC method, stock solution of chlorpyrifos was suitably diluted with chloroform to get concentrations in the linear range of 1-300 ppm. the peak area and retention times were recorded.

The calibration curve for chlorpyrifos was constructed by plotting the ratio of the peak area of chlorpyrifos to against concentration and linearity was evaluated by linear regression equation. The slope, intercept and correlation coefficient values were recorded.

For the UV Spectrophotometric method, calibration graph was prepared with 2.229 to 200 ppm of chlorpyrifos and absorbance was recorded at 290 nm. Each experiment was performed in five replicates.

### 2. 5. Accuracy

Accuracy is a measure of how closely the result of an experiment agrees with the expected result. The difference between the obtained result and the expected result is usually divided by the expected result and reported as a percent relative error. This was calculated by injecting standard solution of chlorpyrifos five times then recording the accuracy of the method.

### 2. 6. Precision

The precision of the method was studied by repeatability (within-day) and intermediate precision (inter-day). The intra-day precision studies were carried out by estimating the response six times on the same day using standard concentration (80 ppm) for HPLC method and 100 ppm for UV spectrophotometric method) of chlorpyrifos and inter-day precision studies were done by repeating the above procedure on three different days. The results of precision studies were expressed as % RSD.

### 2. 7. LOD and LOQ

Sensitivity of HPLC and UV methods were determined from limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated from the calibration curve using the following equations:

$$\text{LOD} = 3.3\sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

where,

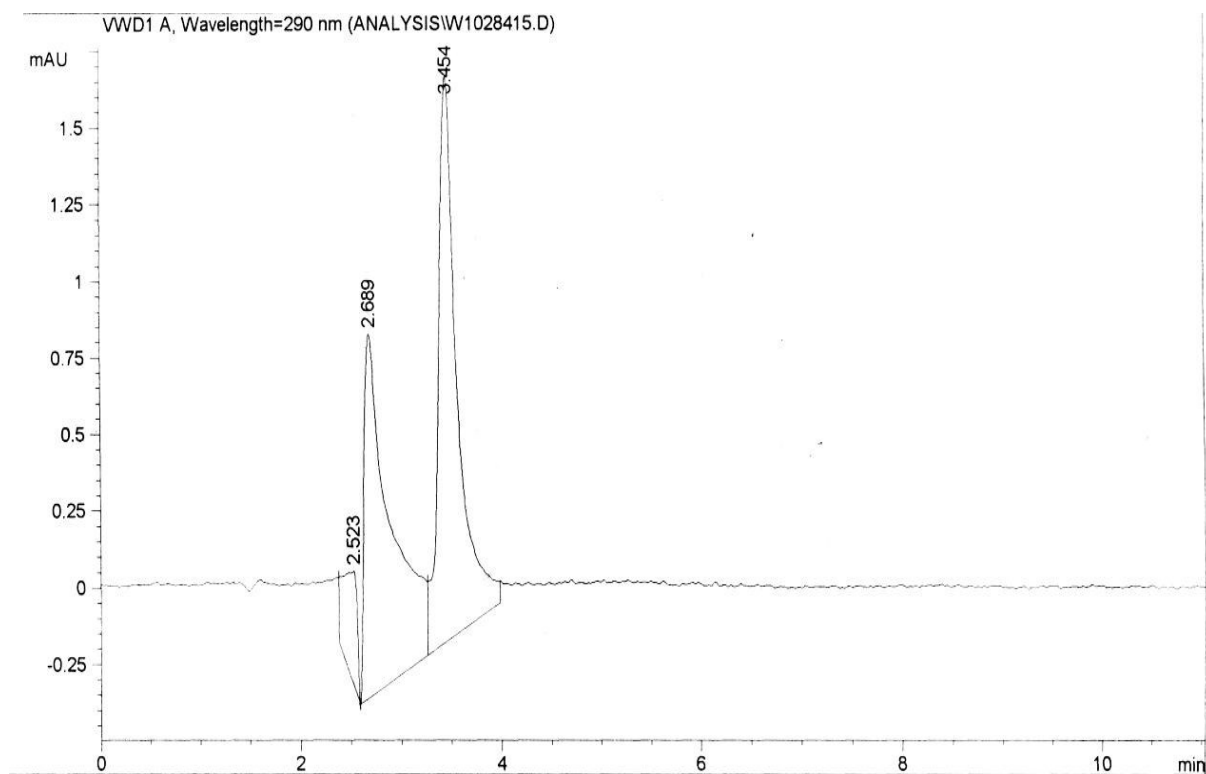
$\sigma$  = standard deviation of y intercept of regression line

S = slope of the calibration curve

## 3. RESULTS AND DISCUSSION

### 3. 1. HPLC Method

The current study describes a HPLC method for the determination of chlorpyrifos. The retention times of chloroform and chlorpyrifos was 2.479 and 3.790 min, respectively. Figure 1 shows the chromatogram of chlorpyrifos.



**Figure 1.** Chromatogram of chlorpyrifos.

The developed method was validated in terms of linearity and range, accuracy, intra-day and inter-day precision, LOD and LOQ. The standard calibration curve showed in Fig. 2 was linear over the concentration range 0.05 to 3000 ppm with a correlation coefficient ( $r^2$ ) 0.9976. LOD and LOQ were found to be 0.05 ppm and 0.5 ppm respectively, indicating the sensitivity of the method. Experiments demonstrated satisfactory accuracy with small relative standard deviations 1.325 (RSD %). The developed HPLC method was precise since the % RSD values were  $< 2$  for both repeatability and intermediate precision studies. RSD of repeatability (intra-day) and intermediate precision (inter-day) ranged from 0.782 to 0.6085 and .9123 to 1.435 respectively. Chromatographic conditions were carefully optimized to get satisfactory resolution between analyte solvent used.

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/ glacial acetic (90/10/0.1, v/v/v) with a flow rate of 1.0 mL/ min. the detection was made at 290 and 230 nm by making preliminary experiment on the chlorpyrifos with using mobile phase composition as solvent on UV-Visible spectrophotometric showed that there was two peaks at 290 and 230 nm by applying the two wavelengths on the HPLC method the using of wavelength 230 nm showed good response with it affected on the LOD to be 0.025 ppm. The proposed methods were also found to be precise and accurate, as depicted by the statistical data of analysis.

### 3. 2. UV-Vis Spectrophotometric method

The solutions were scanned in the wavelength range of 200-1100 nm after making suitable dilutions from the stock solutions. Chlorpyrifos showed absorption maxima at 290 nm. chlorpyrifos in isopropanol as solvent showed linear relationship in the concentration range of 2.229 to 200 ppm with a correlation coefficient ( $r^2$ ) 0.9988. LOD and LOQ were found to be 2.229 6.754 ppm respectively. The % RSD values for intra-day and inter-day precision varied from 0.0531 to 0.0833 and 0.0912 to 0.1485 respectively. spectrophotometric method allowed rapid quantification of chlorpyrifos. Isopropanol was chosen as the solvent because of good solubility, cost and stability.

The spectral characteristics were also good in this solvent. Chlorpyrifos showed absorption maxima at 290 nm which was selected as the detection wavelength since it showed better linearity and sensitivity at this wavelength. Low values of % RSD signify the precision of the method.

### 4. CONCLUSIONS

The validated HPLC and UV-Vis methods were found to be accurate, precise and reliable. UV spectrophotometric method was simpler however HPLC method was more sensitive and the same may be used as an alternative method when advanced instruments like HPLC are not available for routine quantification purpose. Both the methods can be employed for the routine quality control of chlorpyrifos in discharges.

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