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Application of a quechers extraction method for the isolation and identification of selected pesticides by using UV- visible spectrophotometer and TLC

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Abstract

Purpose: The method was developed and validated for Chlorpyrifos using UV spectroscopy with the Quechers extraction application. Method: Chlorpyrifos in the Distilled water and its absorbance were estimated using UV-visible spectrophotometry. Linearity, regression equation, accuracy, precision, standard deviation, etc., parameters were calculated and validated per ICH guidelines. chloropyrifos was determined in marketed form using these validated parameters. Results: The λ (max) of chloropyrifos in the distilled water was 289nm. With a correlation coefficient 0.999, the drug exhibits linearity within the concentration range of 1-6 µg/ml. The method's correctness has been verified, and three distinct recovery experiment levels—50%, 100%, and 150%—were carried out. It was discovered that the recovery percentage ranged from 100.03 to 101.10%. The low values of %RSD indicated the method was precise, accurate, reproducible, and rugged. Conclusion: The above-validated method may be helpful for routine analysis of Chlorpyrifos in a pesticide.

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INTRODUCTION

This area of chemistry uses analytical techniques to guarantee the safety, quality, and purity of pharmaceutical goods. Pharmaceutical analysis in the modern day involves much more than only analyzing formulated drug products (DP), inert substances (excipients), and active pharmaceutical ingredients (APIs). Ensuring the quality of drugs is the principal objective of pharmaceutical analysis. It is common knowledge that a product cannot be tested for quality, yet a DP1 can be made more quality-conscious by carefully planning its testing and using the right approach and equipment. Pharmaceutical

businesses create new APIs through preclinical development and drug discovery research. Drug discovery is associated with creativity and extensive investigation [1]. This endeavor involves the identification and pharmacological activity testing of novel chemical and biological compounds. The effectiveness and toxicity of a promising drug candidate are subsequently examined in animals. The correct dosage is determined by studying the medication candidate's absorption, distribution, metabolism, and excretion—abbreviated ADME. All of these steps—which include preparing, testing, and quantifying the drug candidate as a pure material—involve pharmaceutical analysis, as does testing on blood, urine, and tissue samples. Pharmaceutical companies receive a patent on new APIs, granting them the sole right to manufacture and sell them for a predetermined period [2]. To guarantee efficacy and safety, new APIs must undergo phase I, II, and III human clinical trials before being released into the market.

Once more, pharmaceutical analysis is used, and numerous blood samples from the clinical trials are examined to quantify the new API. Subsequently, a Marketing Application (MA) or New Drug Application (NDA) for Europe or the United Kingdom is created using all of the information from drug development, preclinical development, and clinical trials I, II, and III, and it is submitted to the appropriate regulatory bodies. The European Medicines Agency (EMA) in Europe examines and approves MAs, whereas the US Food and Drug Administration (FDA) handles these tasks in the US. Other drug companies may manufacture and sell active pharmaceutical ingredients as generic drugs without limitations or licenses if a patent does not protect them or if their patent has expired [3].

METHODOLOGY

A -UV SPECTROSCOPY

Objective:

Chlorpyrifos method development and validation were carried out, and the outcomes were recorded. The drug was diluted appropriately from the standard stock solution, and the solutions were scanned within the 200–400 nm wavelength range. This was the analytical wavelength selection process. By using a spectrophotometric approach, the absorption spectra at 289 nm were obtained [4].

Preparation of stock solutions:

After being weighed, 100 mg of Chlorpyrifos was put into a 100 ml volumetric flask and dissolved i n acetic acid. The substance has been thoroughly dissolved and then diluted with distill ed water to achieve the desired final concentratio n of 1000µg/ml.

Analytical concentration range selection and calibration curve creation:

Chlorpyrifos standard stock solution has been pipetted into 10 ml volumetric flasks, and distilled water has been used for dilutions to produce working standard solution concentrations ranging from 1-6 µg/ml. These solutions' absorbance was measured at 289 nm.

It was discovered that the standard solution's analytical concentration range was 1-6µg/ml.

Preparation of sample by QuEChERs extraction method

Weight 30gm of kiwi cut it into small pieces, and prepare the sample in homogenized condition [5].

Add the 5g of sample to a 15 ml centrifuge tube.

Add 5 ml acetonitrile to the centrifuge tube.

Add 4gm of Anhydrous magnesium sulfate and 1gm of sodium chloride to the tube.

Shake the tube vigorously for 1-2 minutes vortex mixer.

Add 1gm of mixed buffer to the tube.

Shake the tube vigorously for 1-2 minutes using a pipette.

Centrifuge tube at 4000 rpm for 5 minutes.

Transfer the supernatant to a clean tube using a pipette.

Add 1 ml of acetic acid to the tube.

Shake the tube vigorously for 1-2 min using a vortex mixer.

Centrifuge the tube at 4000 rpm for 5 minutes.

Transfer the supernatant to a clean tube using a pipette.

Analyze the extract by GC or LC.

Validation:

Validation for the developed spectrophotometric method must have been executed regarding specificity and selectivity, precision and accuracy, linearity, LOD & LOQ according to ICHQ2 (R1) guidelines [6].

Linearity and Range:

Calibration standards spanning the 100-600 µg/ml range were created by dilution-matching the stock solution appropriately.

Plotting the strength of absorbance against concentration yielded the calibration curves.

By employing the least squares approach for linear regression, the calibration line's slope and intercept were found [7].

Accuracy:

To determine the accuracy of this procedure, recovery studies with three distinct concentration levels—50%, 100%, and 150%—were conducted [8].

Precision:

Six replicates of a fixed concentration from the standard stock solution demonstrate the method's repeatability and reproducibility of the estimated sample analysis.

Additionally, the intraday and interday experiment findings were obtained at a confidence interval.

Since the %RSD was found to be less than 2%, the approach seemed to have acceptable precision [9].

Detection of limits (LOD&LOQ):

It is also the littlest concentration of analyte in a sample, which may be identified. However, that may not always be as precise a number as the experimental findings indicate.

The analyte concentration is typically used to express the detection limit. The slope's response and standard deviation are LOD = 3.3 * standard deviation (σ)/s.

The lowest concentration of the analyte in some sample that can then be quantifiable recognized as for relevant precision and accuracy is called the quantitation limit of an analytical approach.

The slope's response and standard deviation are LOQ=10* standard deviation (σ)/s [10].

Robustness:

It illustrates how the analytical technique remains unchanged when minor adjustments are made to the analytical process. Yet, intentional modifications to the method parameters change its dependability when used regularly [11].

Part-B: Thin Layer Chromatography

The number of components in some mixtures can be rapidly determined even by chemists employing TLC, which would be an easy, rapid, and inexpensive method. Whenever a compound's Rf is compared with the Rf of a widely recognized compound, TLC could also be used to help someone's compound's identity inside a mixture (ideally, both drive on the same TLC plate) [12].

Preparation of TLC plates: prepare the slurry using silica gel & distilled water, pour it on TLC plates, and keep it in the Hot air oven for 30 minutes [13].

Sample application: A capillary tube is used to spot extract and standard on a wholly dried TLC plate.

Preparation of mobile phase: Mobile phase-1 is Ammonia and ethanol taken in a 1:2 ratio, and mobile phase-2 is Ammonia and ethanol taken in a ratio of 1:1 [14].

Development of chamber: place the plate into a saturated chamber with the mobile phase. The mobile phase moves upward through the stationary phase due to capillary action.

Visualization of spots: Using a UV chamber to identify the places in TLC [15].

The R_f value

The distance traveled by the compound divided by the solvent's distance traveled is known as the retention factor, or Rf [16].

$$\mathrm{Rf} = \frac{Distance\ Travelled\ by\ the\ Compound}{Distance\ Travelled\ by\ the\ Solvent\ Front}$$

RESULTS AND DISCUSSION:

PART A: UV-Spectroscopy

Overlay Spectrum.

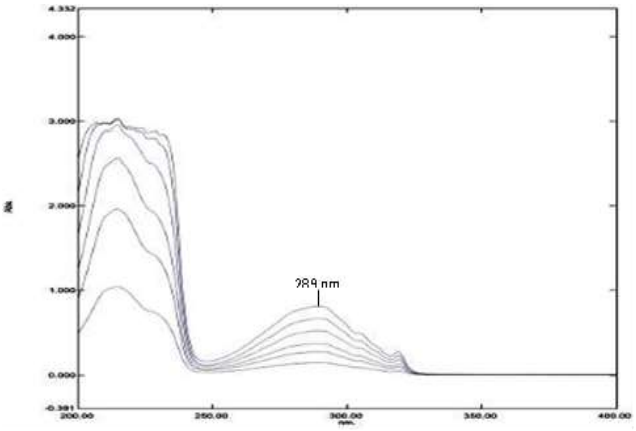

Figure 1 Overlay spectra for Chlorpyrifos showing absorbance at 289 nm

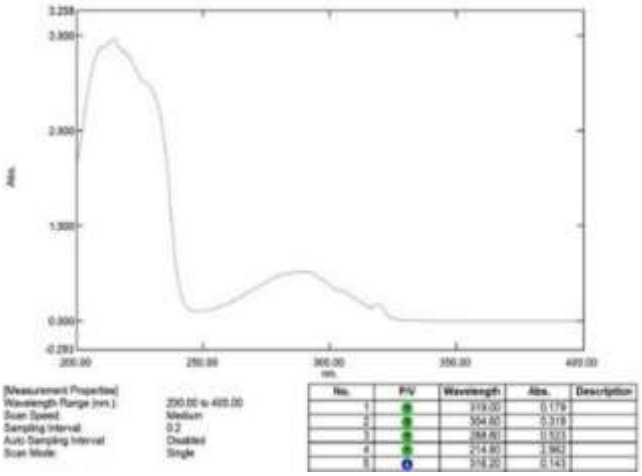

Figure 2 Spectra for Kiwi fruit extract sample Chlorpyrifos showing Abs at 289 nm

ACCURACY

Table 1 Results of Accuracy

Chlorpyrifos					
Recovery Range	Spiked conc. (µg ml^{-1})	Amount added (µg ml^{-1})	Amount found (µg ml^{-1})	% Recovery	% Mean Recovery
50%	2	50.55	50.354	101.10	100.41
			50.825		
			50.472		
100%	4	100.09	100.421	100.09	
			99.579		
			100.281		
150%	6	150.05	150.153	100.03	
			150.153		
			149.847		

PRECISION

Table 2 Precision data

S. No	Intraday (Absorbance)	Interday (Absorbance)
1	0.712	0.718
2	0.714	0.714
3	0.715	0.725
4	0.710	0.721
5	0.712	0.719
6	0.712	0.719
Average	0.7125	0.719
Std. Dev	0.00176068	0.00361478
% RSD	0.25	0.50

DETECTION AND QUANTIFICATION LIMITS

Table 3 Results for Detection and Quantification Limits

Range (µg ml^{-1})	Standard deviation	Linear regression	r2	LOD(µg ml^{-1})	LOQ(µg ml^{-1})
1-6	0.00176068	0.1428x + 0.1341	0.999	0.033	0.100

ROBUSTNESS

Table 4 Result of Robustness

Robust Condition	Parameter	%RSD
Wavelength ± 3 nm	286	0.63
	289	0.49
	292	0.47

PART B: TLC

In Kiwi fruit extract samples were performed TLC with different mobile phases, and their results were obtained,

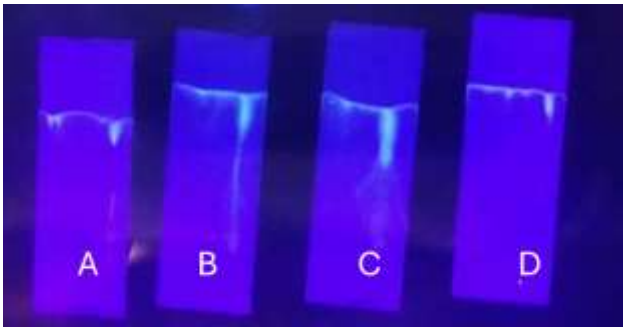

Figure 3 Shows different mobile phases for A: Ammonia and Ethanol(1:1), B: Ethanol and Chloroform(1:1); C: Ammonia and Ethanol(2:1), D: Chloroform:isopropyl alcohol (1:1)

Mobile phase A shows sample extract and pesticide elutes and their Rf value of sample extract 0.75 and chlorpyrifos 0.76.

DISCUSSION OF RESULTS

PART A

VALIDATION PARAMETERS

The developed spectrophotometric method has been validated through specificity and selectivity, precision and accuracy, linearity, LOD & LOQ according to ICH Q2 (R1)guidelines.

Linearity and Range:

Chlorpyrifos was found linear between the 1-6 µg/ml concentration range. Their correlation coefficient was found in the calibration curve to be 0.999.

Accuracy:

The accuracy of the method was checked by performing recovery studies. The recovery studies were determined at three concentration levels: 50%, 100%, and 150%, respectively. The accuracy study found that recovery values of the Chlorpyrifos from the extract were 100.03% to 101.10%, which also implies that the process seems to be accurate. The results obtained are seen in **Table 1**.

Precision:

The method's precision was determined from a single concentration by considering interday and intraday measurements. The %RSD values for interday and intraday were found to be 0.25 &0.50, respectively; the results are shown in **Table 2**.

Detection of limits (LOD&LOQ):

The limit of detection and quantification seems to have been determined by calculating the standard deviation and slope response. The results of LOD &LOQ were tabulated in **Table 3**.

Robustness:

Given that the current approach's robustness shows that modifications to the wavelengths did not significantly alter the analytical results, we may conclude that the method is robust. The %RSD values were found to be 0.63, 0.49, and 0.47 **Table 4**.

PART B

Achieve the isolation of chlorpyrifos from kiwi fruit by employing a mobile phase, which we can observe at short wavelength in a UV chamber, as shown in **Figure 3**.

CONCLUSION

The spectrophotometric method was developed for estimation of Chlorpyrifos in its marketed formulation. The validated parameters like linearity, accuracy, precision, LOD &LOQ was proved as per ICH guidelines. The QuEChERS approach incorporates automation and miniaturisation to facilitate pesticide analysis and cost reduction and simplification of the extraction and purification processes.

Author Contribution

All authors made substantial contributions to the conception, design, acquisition, analysis, or interpretation of data for the work. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all

aspects of the work, ensuring its accuracy and integrity.

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