Full Paper

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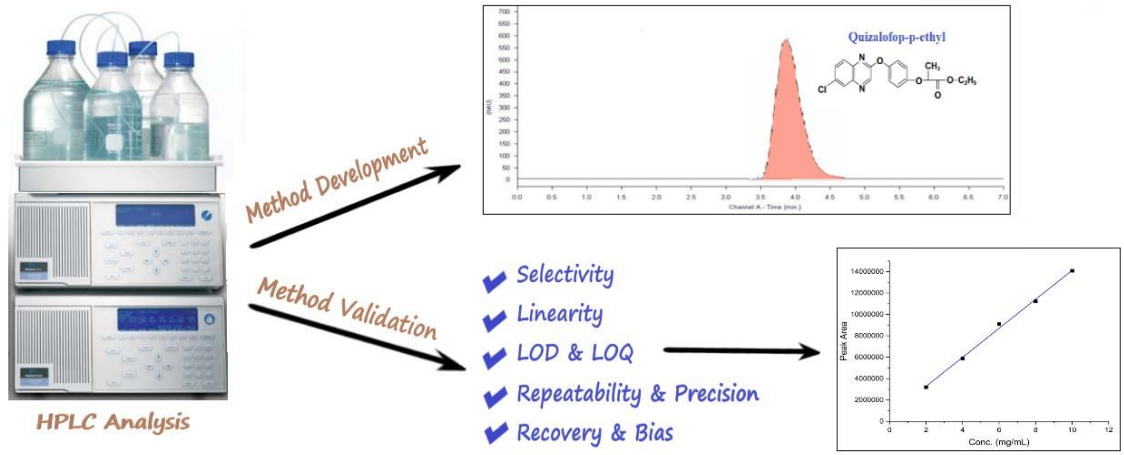
A new Validated Method for Rapid Determination of Commercial Formulations Containing Quizalofop-P-Ethyl by Reversed-Phase Liquid Chromatography

Ismael M. Elkamhawy ✉

Chemistry Department, Faculty of Science, Damanhour University, Damanhour, Egypt

✉ Correspondence to: Ismael.elkamhawy@sci.dmu.edu.eg, Ismaelelkamhawy@yahoo.com



Abstract: A novel quantitative method for the determination of quizalofop-p-ethyl herbicide has been developed and validated using high-performance liquid chromatography with ultraviolet detection (HPLC-UV), which is cost-effective, easy to use, and rapid. This active ingredient is widely used as a systemic herbicide belonging to the aryloxy phenoxy-propionates group and is the active component in Torgy 5% EC® product, a formulation specifically designed for controlling unwanted weeds in various crops. The mobile phase is composed of a gradient of miscible solvents (acetonitrile and distilled water) in the 4:1 (v/v) ratio at a flow rate of 1.5 ml/min with UV detection at 260 nm. The reversed-phase brownlee™ C-18 column that was thermostated at 30°C used for quantification. The method's validation procedures, such as selectivity, linearity, range, LOD, LOQ, and trueness (bias and recovery), were discussed according to ICH guidance standards. The validation results demonstrated that this

innovative method is suitable for assessing the content of quizalofop-p-ethyl within the 2–10 mg/mL range. The high value of the regression coefficient (R^2=0.9971) underscores that the method is fit-for use for analytical applications.

Keywords: Quizalofop-p-ethyl, HPLC, Method validation, LOD, LOQ, Linearity

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Introduction

Pesticides are chemicals that control and reduce the different types of agricultural pests. They are commonly used to improve the quality and quantity of crops [1]. Herbicides are a class of pesticides that are widely utilized. They are used to kill crop plants to make harvesting easier or to destroy target plants that compete with crops [2]. Quizalofop-p-ethyl is a well-known herbicide that has IUPAC name refers to ethyl (2R)-2-[4-(6-chloroquinoxalin-2-yl)oxyphenoxy] propanoate. The empirical formula is $C_{19}H_{17}ClN_2O_4$ and molecular weight is 372.802 g/mol. It is a light-yellow powder compound, slightly soluble in water (0.3 mg/L at 20 °C), highly soluble in benzene (290 g/L) and moderately in ethanol (9 g/L). It is generally stable at pH 7.0 DT^{50} is 3.67 days at 50 °C and DT^{50} 10.7 days at 40 °C. In acidic conditions pH 4 (DT^{50}>1 year at 50 °C), and unstable in alkaline conditions pH 10 (DT^{50} < 2.4 hour at 50 °C) but is unstable in light [3]. The chemical structure is indicated in the Figure 1.

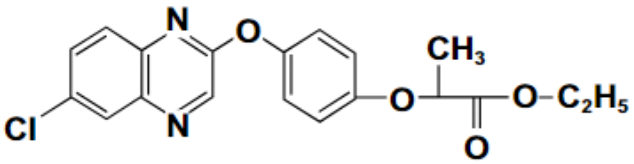

Figure 1. Chemical Structure of Quizalofop-p-ethyl

The importance of this active ingredient (a.i) can be summarized as being responsible for the selective suppression of annual and perennial grass weeds with thin leaves in broad-leaf crops including potatoes, sugar beet, peanuts, cotton, and flax [4-5]. The mode of action of quizalofop-p-ethyl is to block the acetyl-Co-A carboxylase enzyme that causes inhibition of fatty acid synthesis. Also, it is widely used because it has a minimal risk of contamination on nearby sources of water due it only reaches a depth of 10 cm in the soil [6-7]. This a.i. can be formulated as suspension concentrate (SC) and emulsifiable concentrate (EC) herbicide final products [8]. So that, due to the importance of this a.i. there are other analytical methods used for the determination of quizalofop-p-ethyl as literature [9-11] using different techniques, instruments, or mobile phase other than in our innovation.

The chromatography technique has opened up a wide research horizon for scientists to navigate this field and find new methods of analyzing materials of great importance in our daily lives, such as pesticides. One of the most important devices used in this field is high-performance liquid chromatography, commonly known as HPLC, which is an analytical technique used to separate, identify, or quantify each component in a mixture. HPLC is sensitive, specific, and provides fast findings. It can be effectively utilized in the analysis of pesticides. Different types of detectors such as UV, DAD, or fluorescence detectors can be coupled to HPLC. UV detectors are frequently chosen since they are more affordable and readily available [12-13].

It is known that pesticides can have a destructive effect on plants if their concentration exceeds the required range, or they may become ineffective if the concentration is too low. So, there is a strong need to establish new validated methods for determining the actual concentration of pesticides products before released. So that this study aims to find a suitable and facile analytical method to estimate the concentration of this active ingredient content in some selected products on egyptian market such as Torgy 5% EC®, a local Egyptian product produced by Kafr El-Zayat for Pesticides and Chemicals Company which used under study in this research article.

Materials and Instrumentations

Materials

Quizalofop-p-ethyl standard was procured from CPAchem Ltd, India. with a known purity of 97.5% w/w. Acetonitrile HPLC grade was purchased from Scharlab, Spain.

Instrumentations

High-performance liquid chromatography (HPLC) model series 200 PerkinElmer, made in the USA. The pump generates a pressure capacity up to 6100 psi and an ultraviolet detector with a range of 190: 700 nm. Brownlee™ C-18 reversed-phase column (stainless steel, 250 × 4.6 mm i.d., 5 µm particle size) was procured from PerkinElmer instruments, LLC, USA. Data handling output using a computer system with the certified operating system (Windows XP-pack 2) from Microsoft. Distilled water obtained from Merit Water still instrument model W4000. Elmasonic ultrasonic bath used for degassing mobile phase, model S40H, made in Germany.

Parameters of Method Validation

The HPLC validation method was performed according to the International Conference on Harmonization (ICH) guidelines concerning parameters [14]. The validation plan included the limit of detection (LOD), the limit of quantification (LOQ), selectivity, linearity, recovery, and bias. This article presents details on this work.

Experimental

Preparation of mobile phase

The mobile phase consisting of a mixture of (acetonitrile and distilled water) in the 80:20 (v/v) ratio was delivered at a flow rate of 1.5 ml/min with UV detection at 260 nm. The mobile phase was filtered through a 0.45 µm membrane filter, sonicated, and degassed before use by using an Ultrasonic bath. Analysis was performed at room temperature (24±2 ˚C) temperature. Before anything further, chromatography was used to make sure that none of the produced sample solutions contained any interfering peaks. The sample and reference solutions were injected into 20 µL aliquots each.

Preparation of calibration solution

A stock solution was prepared by weighing accurately about 50 mg of quizalofop-p-Ethyl standard substance and diluting it to 25 ml by using acetonitrile. This last step is followed by taking 1 ml and making up the volume to 10 ml with acetonitrile.

Preparation of sample solution

Weigh the equivalent of 50 mg of the substance product (Torgy 5% EC) then diluted to 25 ml by acetonitrile. Complete the next step as a standard preparation solution.

Result and Discussion

Method development

To separate and quantify the pesticide under study, initial analyses used C-8 and C-18 reversed-phase columns, a variety of mobile phase compositions, and various chromatographic configurations. The stationary phase for the separation process was a C18 column with dimensions of 250 mm × 4.6 mm i.d. and a particle size of 5 µm. A 4:1 (v/v) ratio of acetonitrile and distilled water was used as the mobile phase. Analysis was conducted using isocratic elution at a 1.5 ml/min flow rate and UV detection at 260 nm. A 20

µL injection volume was used for all standards and samples. Figure 2 illustrates a chromatogram of quizalofop-p-ethyl using the current developed method.

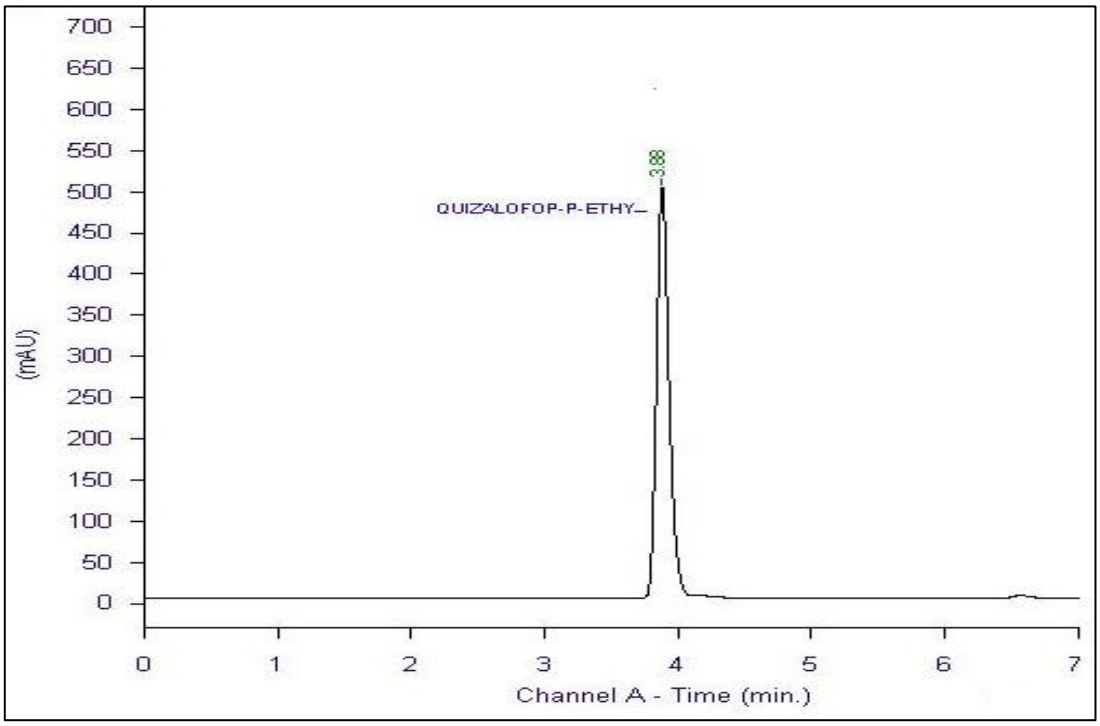

Figure 2. Chromatogram of Quizalofop-p-ethyl pesticide analyzed in this study with a peak at RT 3.88 min.

Method validation

The process of conducting multiple assessments is known as "method validation" and it is intended to confirm that an analytical method is appropriate for the purpose for which it is intended and that it can produce reliable and valuable analytical results. The performance characteristics that will be evaluated to ascertain whether the technique is appropriate for its intended purpose are outlined in the validation plan and the defined procedure for carrying it out [15-16]. ISO/IEC 17025:2017 requires that the laboratory shall validate non-standard methods, laboratory-developed methods, and standard methods used outside their intended scope or otherwise modified [17].

Selectivity

As can be seen from Figure 3, the selectivity of this novel HPLC-UV method was assessed by not detecting peaks or distortions of the baseline at the same retention time for quizalofop-p-ethyl when a blank sample was injected. Then can be observed from Figure 2, the method's selectivity was next proven by spiking a blank sample and monitoring the quizalofop-p-ethyl peak at 3.88 min with no interference from other peaks.

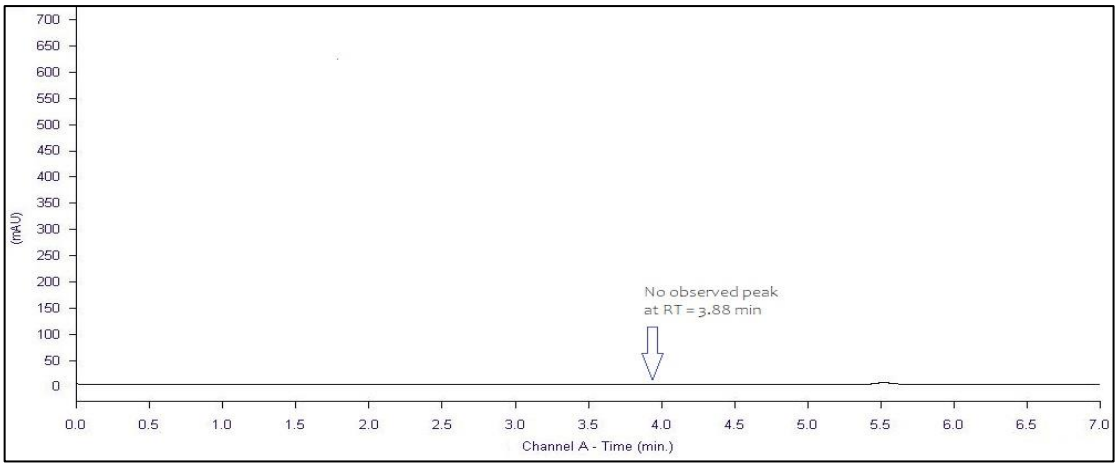

Figure 3. Illustrates the HPLC-UV base line.

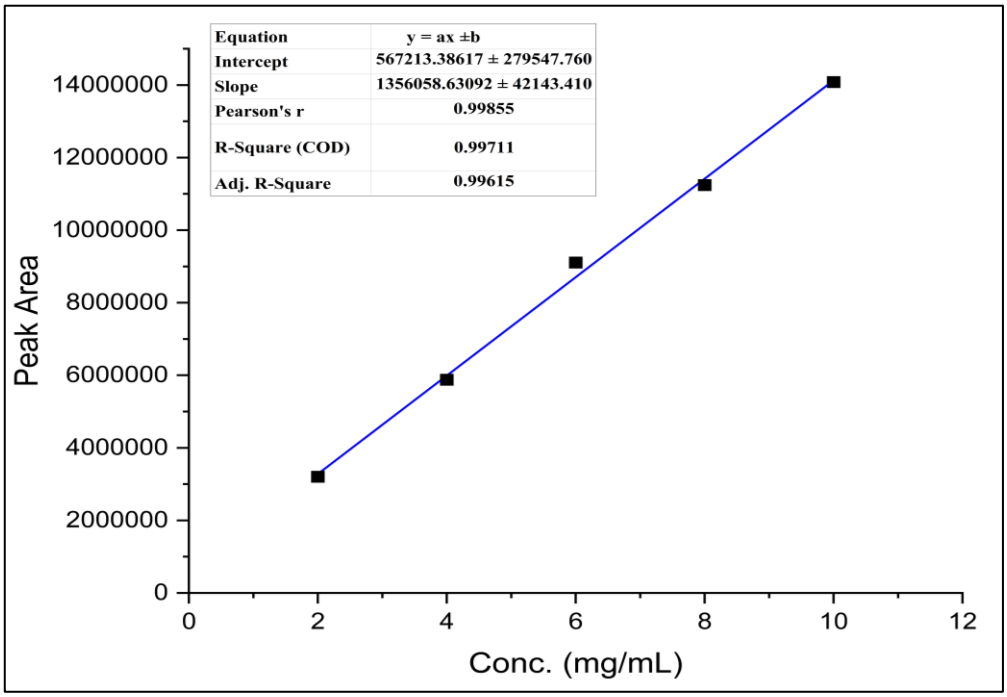

Figure 4. The linear response of peak area against quizalofop-p-ethyl concentration.

Linearity and range

Linearity of an analytical method can be defined as the ability of an analytical method that produces measurement results proportionate to a specified number of calibration points of a calibrant [18]. The value of R^2 is computed to evaluate the linearity of this analytical method and this value must be not less than 0.990 for the method to be valid to use [19-20]. The linear model was expressed by the following equation (1) which shows a relationship between the concentration and the peak area of HPLC-UV and has a slope (a) and intercept (b).

$$y = ax \pm b \qquad (1)$$

The calibration data do not exhibit non-linear trends or outliers, as may be noticed by observing the data (Figure 4). The quality of the regression line was also assessed by the R^2 coefficient, that equals 0.9971. This result showed in Table 1 indicates that this method can be used in a wide concentration range.

Limit of Detection (LOD)

The limit of detection (LOD) was defined as the lowest specified analyte concentration in the matrix that could be identified using the detection of the instrument [21]. From the linearity of the calibration, LOD could be computed according to the following equation (2):

$$LOD = 3.3\sigma/S \qquad (2)$$

where (σ) is the standard error of (X & Y) arrays and (S) represents the slope of the linearity calibration curve. From the previous data, the LOD for the method was found to be 17.8 µg/mL.

Table 1. Results of the linearity study of the HPLC-UV method.

Conc. [mg/ml]	Replicates	Peak Area	Average	STDEV	RSD%
2	R1	3181051.54	3205079.47	20808.80	0.65
	R2	3217087.30			
	R3	3217099.58			
4	R1	5826121.62	5878371.58	63181.82	1.07
	R2	5948591.90			
	R3	5860401.23			
6	R1	9060479.93	9107630.85	47363.43	0.52
	R2	9155203.96			
	R3	9107208.66			
8	R1	11253741.13	11243784.76	14080.44	0.13
	R2	11414182.98			
	R3	11233828.38			
10	R1	14099806.40	14082959.20	73828.92	0.52
	R2	14002162.68			
	R3	14146908.51			

STDEV = Standard deviation, RSD%= Relative Standard deviation

Limit of Quantification (LOQ)

The limit of quantitation (LOQ) was defined as the lowest specified analyte concentration in the matrix that could be identified using the detection of the instrument [21]. From the linearity of the calibration, LOQ was calculated as the following:

$$\mathrm{LOQ} = 10\sigma/S \qquad (3)$$

By implementation the equation (3) the value of LOQ for the method was found to be 54.1 µg/mL.

Repeatability and Precision

Precision is the measure of the repeatability of a method under normal operation. The RSD of the replicates provides the analysis variation and gives an indication of the precision of the test method. RSD for replicate injections should not be greater than 1.5% [19]. RSD% of this method can be estimated by using the following formula (4):

$$\mathrm{RSD}\% = \frac{\sigma}{\bar{x}} \times 100 \qquad (4)$$

Repeatability of this new method was evaluated by calculating the RSD of the peak areas of three replicate injections of standard solutions with five concentrations of 2000, 4000, 6000, 8000, and 10000 ppm which was found to be less than 1.5% as shown in the table (1). These results show that the current method for the determination of quizalofop-p-ethyl is repeatable.

Table 2. Results of the recovery study of the HPLC-UV method.

n	$X_{(Measured)}$ (mg/mL)	$X_{(Avg)}$ (mg/mL)	$X_{(ref)}$ (mg/mL)	Recovery (%)	Bias (%)
1	1.6879	1.71121	1.71	100.07	0.07% (1 ppm)
2	1.7145				
3	1.6904				
4	1.6902				
5	1.6877				
6	1.7301				
7	1.7016				
8	1.7172				
9	1.7490				
10	1.7435				

Recovery and Bias

Recovery is the percentage of the analyte that was fortified or spiked into the test sample before analysis, and it is determined using a method [22]. The recovery of the method was evaluated by measuring a sample ten times at a concentration of 1.71 mg/mL.

The findings were measured and recorded in Table (2). The recovery of the test method can be estimated according to the following equation (5):

$$\text{Recovery (\%)} = \frac{X(Avg)}{X(ref)} \times 100 \quad (5)$$

The bias is computed from these equations (6&7) by the value of ppm and (%) which were found to be 1 ppm and 0.07% respectively.

$$\text{Bias}_{(\text{ppm})} = X(Avg) - X(ref) \quad (6)$$

$$\text{Bias (\%)} = \frac{X(Avg) - X(ref)}{X(ref)} \times 100 \quad (7)$$

Conclusion

The article provides valuable information about a new method for analyzing Quizalofop-p-ethyl, that can now be determined with a precise, simple, accurate, and selective HPLC method. This validated method can be employed for the estimation of this active ingredient in its technical form and emulsion concentrate (EC) formulation. The linearity of the method was conducted in the range of 2000 µg/mL to 10000 µg/mL with an excellent regression coefficient value of R^2=0.9971. The LOQ was found to be 54.1 µg/mL and the observed bias (1 ppm). The validated method can be used by any analytical laboratories for measuring quizalofop-p-ethyl easily.

List of Abbreviations

a.i. Active ingredient
HPLC High-performance liquid chromatography
UV Ultraviolet
i.d. inner diameter
ICH International Council for Harmonisation
DT_{50} Half-life depuration
DAD Diode Array Detector
SC Suspension concentrate
EC Emulsifiable concentrate
LOD Limit of detection
LOQ Limit of quantification
RT Retention time

Acknowledgment

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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