Quantitative Determination of Bifenthrin Contents in Various Formulations of Commercial Samples by Using Newly Developed and Validated Liquid Chromatographic Method

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Abstract The aim of the current study was to develop a unique, fast, proficient, economical, precise and accurate reproducible analysis method for the determination of Bifenthrin which involves the use of reverse phase high performance liquid chromatography with Ultraviolet-Visible detector (HPLC-UV).This method is equally beneficial to detect and quantify the Bifenthrin in multiple modes of formulations available in the market and in raw materials (technical's) with high recoveries to almost 100%. The validation data shows that this analytical method has superb linearity value R^2 = 0.999. Limit of detection (LOD) for this projected analytical method observed was 0.28 mg/L and that the value of LOQ was found to be 0.93 mg/L. This analytical method has shown the higher value of precision (RSD = 0.22%). The accuracy for this intended method has been calculated in terms of recovery percentage which is approximately 100% at optimized conditions. Finally, the robustness results of the decided method have been calculated by changing the rate of flow and the mobile phase ratios of the solvents. The obtained results were found to exist within the allowed accepted figures in terms of Relative Standard Deviation (RSD ≤ 2%). Overall the proposed analytical method can successfully be applied for the quantitative measurement with excellent recoveries percentage in various modes of pesticides products in raw materials and different dosage formulations containing the Bifenthrin as an active ingredient at commercial scale in the laboratory.

Index Terms— HPLC-UV, Bifenthrin, Insecticide, Method Validation

INTRODUCTION

IUPAC name of Bifenthrin is 2-methylbiphenyl-3-ylmethyl (*Z*)-(1*RS,* 3RS)-3-(2-chloro-3, 3, 3-trifluoroprop-1-enyl)-2, 2-dimethylcyclopropanecarboxylate *Roth:* 2-methylbiphenyl-3-ylmethyl (*Z*)-(1*RS*)-*cis*-3-(2-chloro-3, 3, 3-trifluoroprop-1-enyl)-2, 2-dimethylcyclopropanecarboxylate. While the Chemical Abstracts name (2-methyl [1, 1'-biphenyl]-3-yl) methyl 3-(2-chloro-3,3, 3-trifluoro-1-propenyl)-2, 2-dimethylcyclopropanecarboxylate.

While we study the biochemistry, Bifenthrin acts on the nervous system of insects, disturbs the function of neurons by interaction with the sodium channel. Mode of action Contact and stomach action. Effective against a broad range of foliar pests, including Coleoptera, Diptera, Heteroptera, Homoptera, Lepidoptera and Orthoptera; it also controls some species of Acarina. Crops include cereals, citrus, cotton, fruit, grapes, ornamentals and vegetables. Rates range from 5 g/ha against Aphididae in cereals to 45 g/ha against Aphididae and Lepidoptera in top fruit. Formulation types EC; GR; SC; UL; WP. Compatibility not compatible with alkaline materials. Selected products: 'Talstar' (FMC)

Other products may include the 'Aripyreth' (termites) (FMC, Nihon Nohyaku); 'Biflex' (FMC); 'Brigade' (FMC); 'Capture' (FMC); 'Semafor' (FMC); 'Starion' (FMC) (Tomlin & British Crop Protection Council, 2007).

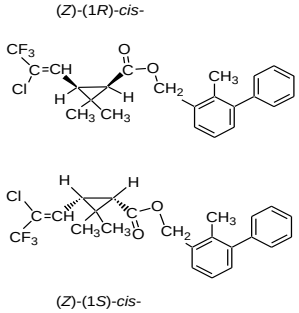

Fig.1. Structural Formula of Bifenthrin Insecticide

Bifenthrin belongs to the pyrethroid family of pesticides. It is oftenly used to kill insects, including fire ants, by affecting the nervous system. It is very toxic to aquatic life. Bifenthrin is also used for household purpose with low concentrations.

Bifenthrin is slightly soluble in water and mostly remains in soil. Bifenthrin usually exists as a white, waxy solid substance with sweet smell. Bifenthrin does not occur naturally but is available in various synthesized forms, like granules, pellets and powder. Bifenthrin has chiral properties with two main enantiomers like 1S-cis-bifenthrin and 1R-cis-bifenthrin. 1R-cis-bifenthrin is the best insecticide. It is approximately 300 times more effective than 1S-cis-bifenthrin**.** (Liu *et al.*, 2008).

Bifenthrin toxicity studies revealed two types of pyrethroid compounds "with and without α-cyanogroup". Alpha-cyano pyrethroids permanently block sodium channels in mammals and insects. Pyrethroids that do not have an alpha-cyano group such as bifenthrin temporarily binds sodium channels. So, bifenthrin pyrethroid does not affect the resting potential. In comparison to other pyrethroids, bifenthrin will cause the opening of sodium channel for a short period of time. The mechanism is almost same in mammals and invertebrates but much less effect shows on mammals due to low affinity of Bifenthrin with sodium channels, higher body volume and high body temperature (Lund & Narahashi, 1983).

The half-life of bifenthrin in soil, water and air at different pH values and temperatures was studied under aerobic or anaerobic conditions (Keith & Walker, 1992). Bifenthrin has low solubility in water and is not volatile. Bifenthrin is effective against malaria, it can be used to treat mosquitoes and on interior walls to get rid of mosquitoes. (Al-Amin *et al.*, 2011; Hougard *et al.*, 2002). The LC50 value of bifenthrin is 17 mg in Bees (Dai *et al.*, 2010). Since bifenthrin has good biodegradability, it is not more toxic to humans and animal especially the Mammals. The U.S. Environmental Protection Agency has classified bifenthrin as a class C substance that may be carcinogenic to humans because it causes bladder tumors in mice and adenocarcinoma in liver of male mice.

Bifenthrin was found to be most effective against large red ants. Bifenthrin is also taken to be very effective against pests like fleas, spiders, aphids, beetles, flies, thrips, mites, moths, aerial birds, ticks, yellow jackets, grubs and grasshoppers. It is often used in nurseries, orchards and homes. The most common use of bifenthrin is in corn. In the United States, 70% of hops and raspberries are treatd with bifent hrin. Bifenthrin is also used in the textile industry to protect woolen products from insects. Bifenthrin is considered the best alternative of permethrin (Ingham *et al.*, 2012). Due to the high use of bifenthrin in plants, management of bifenthrin is very important. To ensure this, the percentage of bifenthrin as recommended on the label must be within the recommended range of percentage. Various methods have been developed and reported in the literature for the determination of bifenthrin in different formulations. Quantitative studies on the active ingredient of bifenthrin in residues and various formulations often use different chromatographic and spectroscopic techniques. HPLC is used to determine the residue of bifenthrin in biodiesel nanoemulsions in wastewater, cotton, soil, water and to study the enantio mers of bifenthrin (Hamid *et al.*, 2016; Liu *et al.*, 2005; SHARMA; Ali *et al.*, 2015; Fan *et al.*, 2014; Harwood *et al.*, 2013; Wang *et al.*, 2007; Yan *et al.*, 2019; Snake & Goby, 2012). GC-ECD, GC-FID and GC-MS was also used for Bifenthrin residues determination in soil, water, waste water, plants, foods and feeds (Zhang *et al.*, 2020; Balakrishnan *et al.*, 2009; Rao *et al.*, 2014; Styarini *et al.*, 2014; Kikta Jr & P, 2011; Nazir *et al.*, 2020; Hamid & Hamid, 2015; Wei, 1991; Zhang *et al.*, 2017). ELISA was also used for Bifenthrin study (Bo *et al.*, 2008). Combined techniques like IR, NIR Spectroscopy, SEM, TEM and UV-Visible were also used in studies of Bifenthrin (Meder & Ebdon, 2013; Szajnecki & Gawdzik, 2019; Yang *et al.*, 2016). TLC, HPLC and GPC were also used in studies if bifenthrin (Food & Residues, 1997). Bifenthrin was also studied by HPLC/RAM (Putt, 2005).

All of the above methods and techniques were used to analyze Bifenthrin. The running cost of all the above mentioned analytical methods for the analysis of the Bifenthrin in different formulations is high. So, these analytical methods are costly and not suitable economically for the local industry to adopt. Most of the local industry has the facility of the HPLC-UV in their quality control laboratories and they need the most suitable and economically favorable analytical method to be used in their quality control Labs for the analysis of bifenthrin active ingredient both in the various types of formulations and in the raw materials. Therefore, the current work was done to develop a unique HPLC-UV chromatographic analytical method for the quantitative measurement of bifenthrin in various types of formulated products and raw materials which should be the most simple, economical, quick, repeatable, reproducible but with same value of accuracy and precision. This method is most suitable and can easily be adopted on commercial scale by any quality control laboratory to analyze bifenthrin contents in local and/or anywhere in the world at commercial level.

EXPERIMENTAL

Reagents and Chemicals

The highest purity HPLC gradient grade chemicals were used throughout the experimental and practical work. Acetonitrile from Duksan Pure Chemicals Korea, Water from VWR Chemicals and bifenthrin analytical standard of Known Purity 98% from Chem Services USA were

purchased. A sample of bifenthrin 10% EC (Emulsifiable Concentrate) product marketed by name of Charm was collected from Solex Chemicals Quality Control local laboratory. From the local market of Multan, Pakistan some other samples of 10% EC (Emulsifiable Concentrate) were arranged.

Apparatus

Filtration assembly from Glasco with vacuum pump was used for mobile phase filtration. Filter paper of 0.25 µm from Sartorius was used for filtration of mobile phase. Ashless filter paper (42 No.) from Sartorius was used for filtration of standard and sample solutions. Weighing was done by using a highly sensitive Analytical weighing balance ranges from 0.01 gm – 220 gm (Mettler Toledo). An ultra sonic water bath (GT Sonic D3 China) was used. Certified glass wares from Iwaki Pyrex were used throughout the experimental work.

Shimadzu Japan HPLC system consisting of LC-20AT pump and SPD-20A UV-VIS detector was used. An HPLC system of 10 AT of SPD -10A VP UV-VIS detector was also used. A zorbax 250 mm x 4.6 mm (i.d) packed C18 column with 5µm (particle size) from Agilent Technologies was purchased and used. Isocratic elution was performed for the separation of bifenthrin contents by using the mobile phase (Acetonitrile 90% + Water 10%). Flow rate used during the analysis was 2 mL/min. The analyte volume injected was 20 µl. Micro glass syringe with stainless steel piston of 50 µl was purchased from SGE. The wavelength was used 230 nm for the detection of bifenthrin. The content %age of the bifenthrin sample was detected by comparison of retention time of the analyte peak with the retention time of analytical standard peak. The bifenthrin peak was detected at about retention time of 6.1 minutes.

Preparation of Analytical Standard Stock Solution

Stock solution of bifenthrin standard was prepared by taking 0.25 ± 0.0001 gm of pure analytical standard of 98% purity into a separate 50 mL volumetric flask. The analytical standard of bifenthrin was dissolved into the 10 mL of diluent (Acetonitrile 90% + Water 10%) by sonicating moderately and then cooled to room temperature and the volume was made up to 50 mL with mobile phase. The standard solution was shaken vigorously for homogenization. This solution of analytical standard was found to be stable for 24 hours.

Preparation of Calibration Standard Solutions

Working standards of bifenthrin 25, 50, 75, 100 and 125 mg/L from standard stock solution for linearity curve were prepared by diluting up to the mark 50 mL with diluent (Acetonitrile 90% + Water 10%). Filter all the working standards solutions with membrane filter paper of 0.45 µm. The HPLC data was recorded on the chromatograms after HPLC analysis and percentage recovery was calculated. Three repeated readings were taken.

Preparation of the Bifenthrin Product Sample Solution

25 mg of bifenthrin contents from each type of various formulations were prepared individually into separate 50 mL volumetric flasks. The volume was made up with diluent (Acetonitrile 90% + Water 10%). The sample solution was vigorously shaken. The sample with membrane filter paper was filtered and maintained it at room temperature for analysis on HPLC and the data was recorded on chromatograms.

2.6 Proposed Method

HPLC-UV system was used where detector wavelength was 230 nm and column was C18 Zorbax Agilent Technologies. The Mobile Phase used was Acetonitrile 90% + Water 10%. The Flow rate was maintained at 2.0 mL/minute and retention time was found to be 6.1 minutes.

The bifenthrin contents were calculated by using equation "Eq. (1)".

$$Bifenthrin\ contents\ \%\ \left(\frac{w}{w}\right) X1 = {}^{A_2\ x\ m_1\ x\ P}/_{A_1\ x\ m_2} \qquad (1)$$

Bifenthrin Content % (w/v) = Bifenthrin (w/w) x Density of Bifenthrin Liquid

Where

A_1 = Average peak area of the Bifenthrin in the standard solution

A_2 = Average peak area of the Bifenthrin in the sample solution

m_1 = mass of Bifenthrin standard (mg)

m_2 = mass of Bifenthrin sample (mg)

P = Purity of Bifenthrin analytical standard

Graphical scheme of experimental work has shown in Fig. 2.

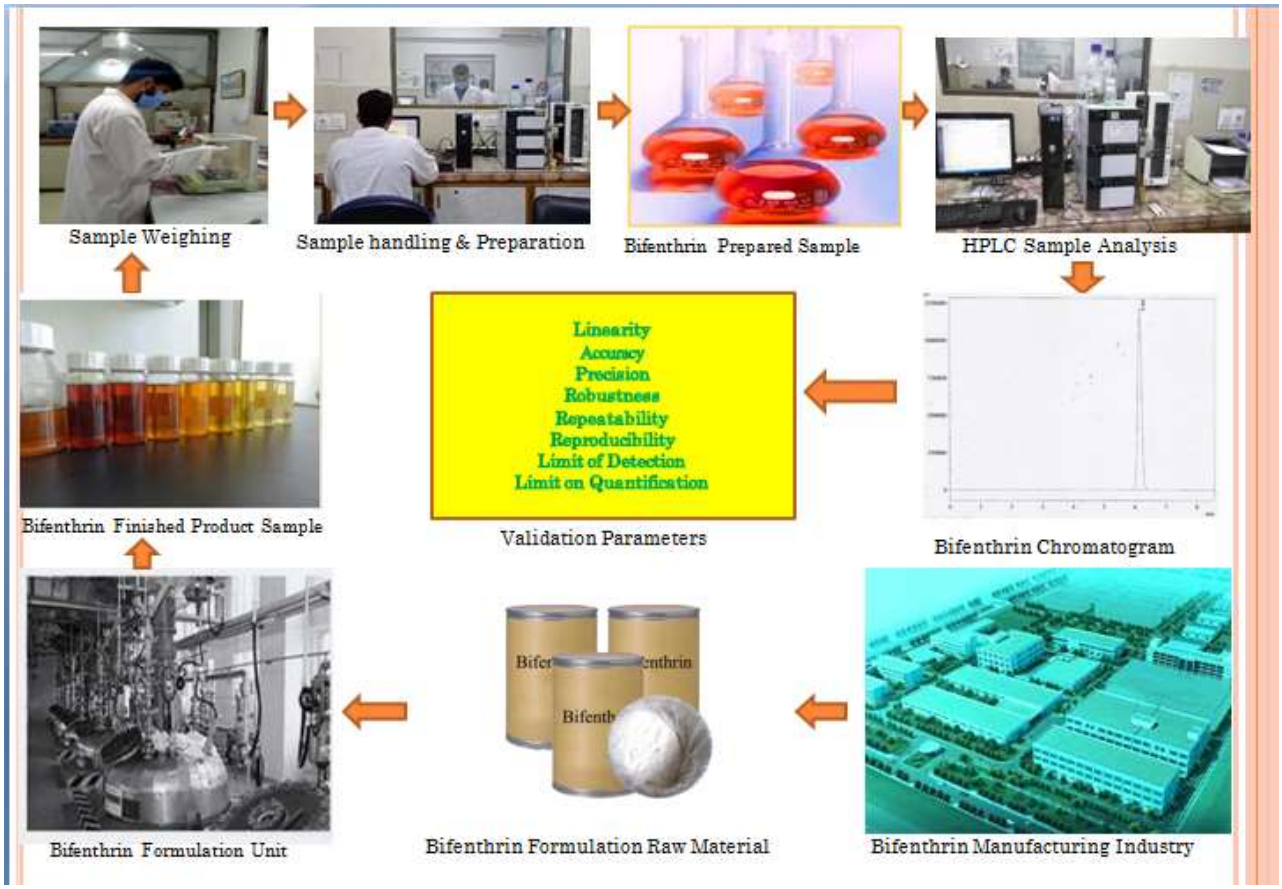

Fig. 2. Graphical scheme of the experimental work

Validation of the Method

Method validation of bifenthrin was done with following parameters in accordance with the ICH guidelines. These parameters are system suitability, precision accuracy, linearity, range, LOD, LOQ selectivity, robustness reproducibility and repeatability.

Linearity

Linearity of the freshly developed method was achieved with five concentration level of Bifenthrin analyte by plotting the graph between the peak area and the concentration.

System Suitability

The suitability of the system was tested by performing five consecutive injections of every type of the formulation under optimized conditions. The system suitability test was conducted on everyday of validation and found it within the range of accepted criteria.

Specificity/Selectivity

To study the interfering effects of inert materials with the peak of Bifenthrin analyte all types of products were tested invidiously. It was found that the inert materials have no conflicting effect to the Bifenthrin analyte chromatographic responses.

Precision

Inter and intraday analysis were performed to confirm the precision to evaluate the inter and intraday precision, analysis of four various kinds of formulations of Bifenthrin were used. The percentage relative standard deviations for the three consecutive day results were calculated by the following formula:

% RSD = (SD/Mean x 100)

SD = Standard Deviation of analyte

Mean = Average of analyte readings

Repeatability

The repeatability of the developed method was calculated in form of percentage of relative standard deviation (%RSD) of the five consecutive readings in an intraday analysis.

Reproducibility

The reproducibility of the developed method was calculated with respect to the instrument in terms of accuracy of five replicate reading obtained and was demonstrated in the form of percentage of relative standard deviation (%RSD).

Limit of Detection and Quantification (LOD & LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for the developed method of the Bifenthrin analytes were calculated by the given formula.

LOD = 3 α/S

LOQ = 10 α/S

α = the standard deviation of the response

S = the slope of the calibration curve

Robustness

The ruggedness and robustness of the developed method was also calculated by changing the mobile phase ratios and the flow rates keeping all the other factors constant like the wavelength of the detector and the column.

Results and Discussion

Optimization and Method Development

To determine bifenthrin in various types of formulations in a consistent quantitative way, the current analytical method was developed. This developed analytical method is cost effective contains HPLC-UV with optimized chromatographic conditions. The parameters were checked for optimization and also the validation of the developed method was carried out.

Validation parameters like the asymmetric peak, low resolution and merging of chromatographic peak of bifenthrin analyte with co-extracts of inerts in the sample of formulations showed variations. Symmetric peak with excellent separations was attained at the mobile phase ratio of Acetonitrile : Water :: 90 : 10 at flow rate of 2 mL as shown in Table 9 and at the λ_{max} 230 nm.

Method Validation

The parameters used to validate analytical method were suitability, precision accuracy, linearity, LOD, LOQ, range, selectivity, repeatability, robustness, and reproducibility. The run time of the method was programmed at 10 minutes to elute all the inerts used in formulation types and to reproduce the stable and smooth baseline while the retention time was found to be about 6.10 minutes.

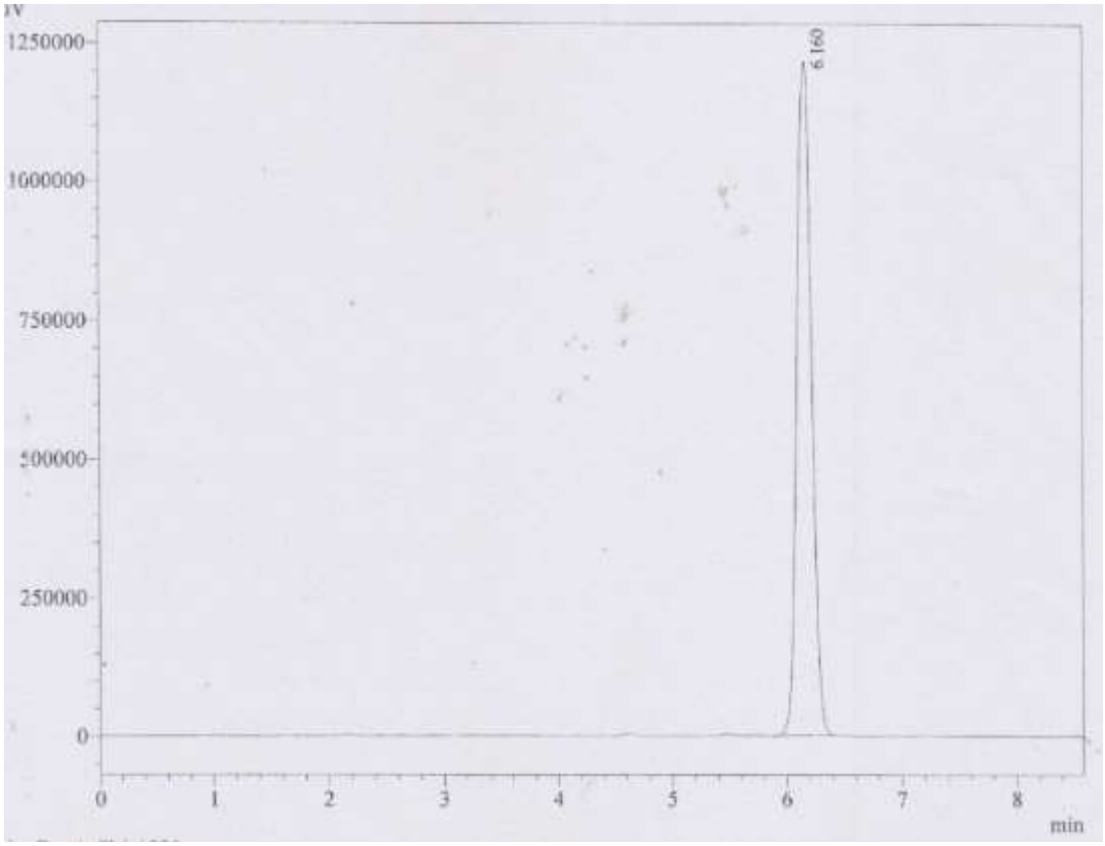

Fig. 3 (a) HPLC chromatogram of the Bifenthrin standard solution

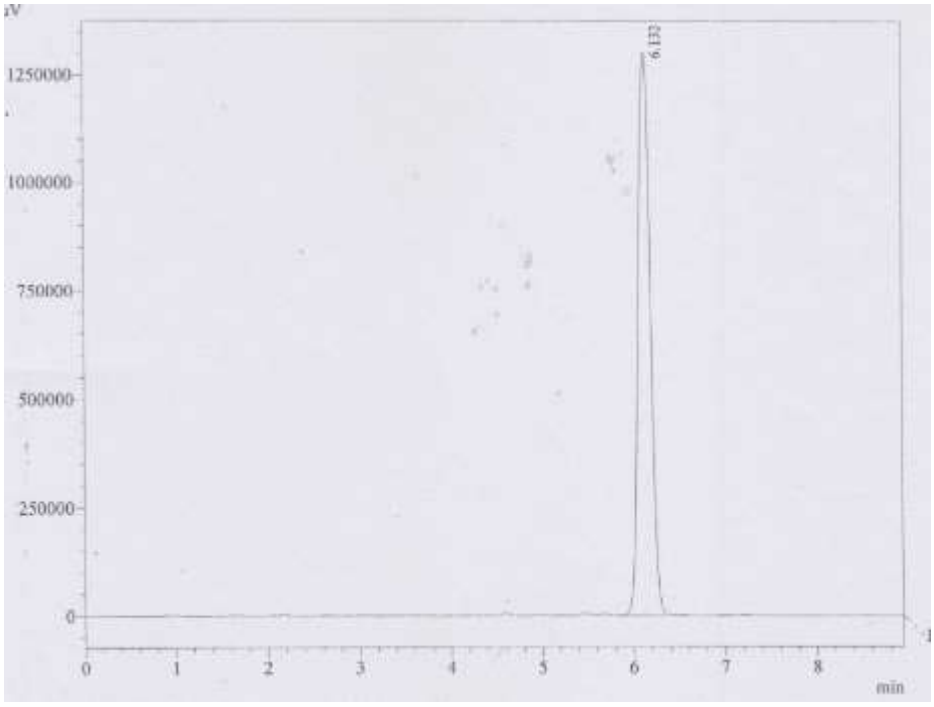

Fig. 3 (b) HPLC Chromatogram of the Bifenthrin sample solution

The HPLC chromatograms of Bifenthrin in Fig. 2 (a) and 2 (b) showing the same retention time (6.1 minutes) in analytical standard solution as well as in sample solution.

Linearity of Bifenthrin

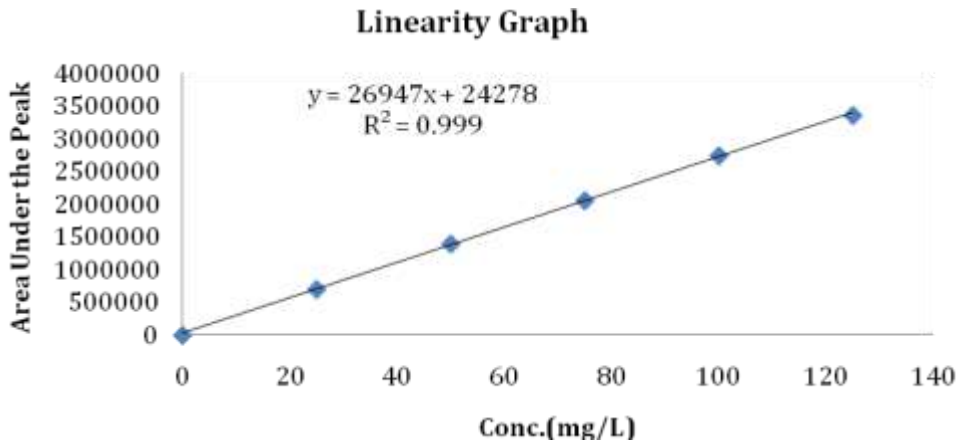

Fig. 4 Calibration curve of Bifenthrin by the proposed method

Fig. 4 is showing the linearity curve was plotted between the concentration and the peak areas. The Linearity of the method developed which was evaluated by using different concentrations of 25, 50, 75, 100 and 125 mg/L per 50 mL. The value of correlation coefficient (R^2) in straight line equation obtained was $R^2 = 0.999$. The R^2 value shows that the solubility is verified by this HPLC method for bifenthrin pure active ingredient.

Precision for Bifenthrin

Table 1: Precision of the developed method for determination of Bifenthrin

	Area of Standards Bifenthrin
1	10873978
2	10919152
3	10930050
4	10902594
5	10933916
Average	10911939
S.D	24451
RSD%	0.23

For the system suitability, the value of relative standard deviation is (RSD = ± 2%). For bifenthrin the value of RSD% obtained was 0.22% of the five replicate readings (Table 1).

Selectivity for Bifenthrin

Table 2: Specificity of the developed method for Bifenthrin

Formulation	Results in Mixture	Mean Result in Soul Sample		Recovery (80% – 120%)	Remarks
		Peak area of the standard solution	Peak area of the sample solution		
Bifenthrin	10.00%	10911938	12095724	99.90%	Pass
		9.99%			

The method is specified and selective for Bifenthrin active ingredient contents which were monitored by the use of blank sample and analyte standard solution separately, in which no peak was observed and detected near the peak of desired analytes. So, the method proved to be highly specific and selective (Table 2).

Accuracy for Bifenthrin

Peak areas (triplicate measurements) for five different concentrations of nitenpyram in standard and the sample solutions (25, 50, 75, 100 & 125 mg/L) were measured under the optimized instrumental conditions. Percentage recoveries are given in Table 3.

Table 3: Area under the peak of sample for the accuracy of the method developed for the Bifenthrin

Conc.(mg/L)	Standards		Peak Area	Peak Area Mean
25	A	A1	700025	703845
		A2	710253	
		A3	701255	
50	B	B1	1394546	1386566
		B2	1379457	
		B3	1385690	
75	C	C1	2056126	2058333
		C2	2060550	
		C3	2058327	
100	D	D1	2743187	2759582
		D2	2785222	
		D3	2750337	
125	E	E1	3357522	3359095
		E2	3359242	
		E3	3360520	

Table 4: Accuracy of the developed method for determination of Nitenpyram

Known Conc. of Bifenthrin (mg/L)	Mean (Peak Area) of Bifenthrin Sample solution	Mean (Peak area) of Bifenthrin Standard solution	Measured Conc. of Bifenthrin in sample solution (mg/L)	Recovery of Bifenthrin (%)
25	703845	700024	25.14	100.55
50	1386566	1394021	49.73	99.47
75	2058333	2056070	75.08	100.11
100	2759582	2743199	100.60	100.60
125	3359095	3357352	125.06	100.05

Standard solutions of bifenthrin at various concentrations 25, 50, 75, 100 and 125 mg/L for the accuracy of the method developed the peak areas of the above said concentration was calculated and linearity curved was drawn.

Repeatability for Bifenthrin

Table 5: Repeatability of the developed method for determination of Bifenthrin

Readings	Bifenthrin peak area
01	12088304
02	12097149
03	12103222
04	12074691
05	12115254
Mean	12095724
S.D	15306
RSD%	0.13%

In evaluating the repeatability parameter for the Bifenthrin developed method it had been observed that by analyzing the Bifenthrin analyte within different interval of times upon same conditions and instruments, the results showed the RSD% do not deviate the standard value (RSD% ≤ 2%).

Reproducibility for Bifenthrin

Table 6: Reproducibility of the developed method for Bifenthrin

Reading	Bifenthrin (Peak Area)	
	HPLC (20AT)	HPLC (10AT)
01	12088304	5669113
02	12097149	5667220
03	12103222	5668492
04	12074691	5670193
05	12115254	5648250
Mean	12095724	5664654
S.D	15306	9233
RSD (%)	0.14%	0.17%

Table 7: Reproducibility of the developed method for determination of Bifenthrin

Commercial Formulation	Company	Proposed Method	
		Recovery %age	RSD (%)
Charm 10% (EC)	Industry A	100.40%	0.52
Bifenthrin 10% (EC)	Industry B	99.20%	0.25
Bifenthrin 10% (EC)	Industry C	99.60%	0.46
Surprise 25% (SL) (Bifenthrin 10% + Nitenpyram 15%)	Industry D	100.02%	0.35
Bifenthrin 10% (EC)	Industry E	100.30%	0.48

While performing the reproducibility parameter on two HPLC instruments, it had been observed that the developed method for the bifenthrin analyte did not deviate the standard value of (RSD% ≤ 2%) while performing the same bifenthrin analyte on another instrument HPLC LC-10AT. Hence, the developed analytical method found fit for analyzing bifenthrin contents in various formulations at commercial scale.

Limit of Detection (LOD) and Limit of Quantification (LOQ) for Bifenthrin

Table 8: Limit of Detection (LOD) and Limit of Quantitation (LOQ) values of the developed method for Bifenthrin

Readings	Bifenthrin (mg/L)
1	50.84
2	50.80
3	50.74
4	50.68

5	50.70
Mean	50.72
SD (So)	0.065
Śo=SQR(2)* so	0.10
LOD=3* Śo	0.30
LOQ=10* Śo	1.00

Table 8 shows that the value of LOD for Bifenthrin was found to be 0.30 mg/L and that the value of LOQ was found to be 1.00 mg/L which is the clear indication of signal-to-noise ratio 3:1.

Robustness for Bifenthrin

Table 9: Robustness of the developed method for determination of Bifenthrin

Sample No.	Change of Flow Rate			Change of Mobile Phase		
	Peak area at 1.8 mL/min	Peak area at 2.0 mL/min	Peak area at 2.2 mL/min	Acetonitrile : Water 95 : 05	Acetonitrile: Water 90 : 10	Acetonitrile : Water 85 : 15
1	13343282	12088305	11936597	10107315	12088306	16414206
2	13346791	12097150	11967937	10048685	12097150	16336287
3	13331780	12103221	11977683	10113518	12103223	16421043
4	13290534	12074692	11976971	10059969	12074692	16416420
5	13314712	12115253	12000152	10117343	12115255	16432565
Mean	13325421	12095725	11971869	10089367	12095723	16404104
Std. Deviation	23174	15306	23019	32432	15306	38568
RSD %	0.17%	0.13%	0.19%	0.32%	0.13%	0.24%

While performing robustness of the method developed for the Bifenthrin it had been observed that by increasing the flow rate of mobile phase from 2.0 mL/minute to 2.2 mL/minute the area under the peak became decreasing. While the RSD% was remained within the prescribed limit of RSD% ≤ 2%. While decreasing the flow rate of mobile phase from 2.0 mL/minute to 1.8 mL/minute the area under the peak became increasing. In this case again the RSD% did not deviate from the standard value. Similarly robustness of the method had been evaluated by changing the mobile phase concentrations from (ACN : Water = 90 : 10) to (ACN : Water = 85 : 15) the area under the peak became increasing but the RSD% do not deviate the prescribed standard value. During the decrease of water ratio in the mobile phase (ACN : Water = 90 : 10) to (ACN : Water = 95 : 05) the area under the peak became decreasing but again the RSD% shows no deviation from the standard value. So, it has been proved that the developed analytical method for bifenthrin contents is found to be robust.

Summary of the validation parameters of developed method for the Nitenpyram

Table 10: Summary of the validation parameters of developed method for the determination of Bifenthrin

Parameters	Results (Bifenthrin)		Acceptance Criteria
Linearity	Correlation Coefficient = 0.999		Correlation Coefficient NLT* 0.97
Precision	0.22% RSD		% RSD NMT* 2.0
Accuracy	**Concentration (mg/L)**	**% Recovered**	% Recovery within 80% - 120%
	25	110.60%	
	50	99.50%	
	75	99.99%	
	100	100.65%	
	125	99.99%	
Repeatability	0.13% RSD		RSD ≤ 2.0%
Reproducibility	**HPLC – 20AT**	**HPLC – 10AT**	
	0.13% RSD	0.16% RSD	
Detection and Quantitation Limit	**LOD**	**LOQ**	-
	0.28 mg/L	0.93 mg/L	
Robustness	**Change**	**% RSD**	% RSD NMT 1.5
	Flow 1.8 mL	0.17%	
	Flow 2.0 mL	0.13%	
	Flow 2.2 mL	0.19%	
	(Mobile Phase) Acetonitrile : Water 95 : 05	0.32%	
	Acetonitrile : Water 90 : 10	0.13%	
	Acetonitrile : Water 85 : 15	0.24%	

* Not Less than in accordance to the ICH Analytical procedures developments Guidelines (Guideline, 2022)

+ Not More than in accordance to the ICH Analytical procedures developments Guidelines (Guideline, 2022)

In Analytical research the development of the methods for the determination of analytes is extremely important. Development of easy, efficient, low cost, repeatable and reproducible analytical methods both for the drugs and pesticides by HPLC are always demanding in industrial research (Hajare et al., 2016). This study proposed a facile, efficient and simple analytical method for the determination and quantification of Bifenthrin contents by HPLC both in raw material and pesticide dosage formulations in quality control sector of the industries. Analytical standard solution for the Bifenthrin contents was analyzed. Optimization of parameters for the solvents for the mobile phase and sample & standard solutions has also been done which showing the excellent recovery results (almost 100%) for the analytes. The Chromatograms were showing the similar retention time both for the sample and standard which showed the harmony of the analytical method on the basis of the parameters initially optimized, the method is successfully validated by considering the parameters like the

linearity, precision, accuracy, repeatability, reproducibility, suitability of the system, detection limit, quantification limit, specificity and robustness. In analytical method validation, the parameter of the linearity is taken as the first step (Saleh et al., 2021).

System Suitability

The suitability of the system was tested by performing five consecutive injections of every type of the formulation under optimized conditions and found within the range of accepted criteria.

Table 11: Intra-day and Inter-day precision

		Intra-day Analysis		Inter-day Results	
Sr.#	**Formulation Type**	***Results**	**%RSD**	***Results**	**%RSD**
1	Bifenthrin 10% EC	10.01%	0.40%	10.05%	0.28%
2	(Bifenthrin 15% + Nitenpyram 10%) 25% SL	15.04%	0.17%	14.97%	0.14%
3	Bifenthrin 10% WP	10.03%	0.30%	10.04%	0.23%
4	Bifenthrin 10% EW	9.98%	0.32%	10.08%	0.30%
5	Bifenthrin 20% WDG	19.99%	0.15%	19.97%	0.19%
6	Bifenthrin Tech 96% TECH	95.97%	0.10%	95.97%	0.09%

(*) Average of 5 replicates

Table 12: Inter Laboratory Comparison Test for multiple pesticide formulations

		LAB A		LAB B		LAB C	
Sr.#	**Formulation Type**	***Results**	**%RSD**	***Results**	**%RSD**	***Results**	**%RSD**
1	Bifenthrin 10% EC	9.99%	0.25%	10.01%	0.32%	10.03%	0.21%
2	(Bifenthrin 15% + Nitenpyram 10%) 25% SL	15.01%	0.09%	15.03%	0.28%	15.01%	0.09%
3	Bifenthrin 10% WP	10.02%	0.22%	9.98%	0.19%	10.03%	0.21%
4	Bifenthrin 10% EW	10.03%	0.33%	9.99%	0.23%	10.01%	0.43%
5	Bifenthrin 20% WDG	20.01%	0.11%	20.02%	0.25%	20.03%	0.08%
6	Bifenthrin Tech 96% TECH	96.03%	0.13%	95.98%	0.09%	96.02%	0.12%

(*) Average of 5 replicates

LAB A: Solex Chemicals Quality Control Laboratory Multan.

LAB B: Exin Quality Assurance Laboratory Multan.

LAB C: Hexon Quality Assurance Laboratory Multan.

In this study, the range of the precision was in acceptable limits was better for this analyte than the methods reported previously. Accuracy of the validated method was showing the excellent results. Percentage recovery of the Bifenthrin was also calculated for every concentration by comparing the area under the peak of the standard solution and sample solution. The obtained results showed that the recovery percentage was maximum at the concentration 25 mg/L (100.88%) while at 50 mg/L (99.47%), 75 mg/L (100.11%), 100 mg/L (100.60%) and 125 mg/L (100.05%). So, the proposed method for the Bifenthrin showed the excellent results with excellent recoveries at different concentrations. So, under the optimized conditions it has been proved that the method developed for the Bifenthrin by HPLC was accurate and reproducible for different types of sample with excellent recoveries under the optimized conditions.

At the end, the evaluation of the robustness was done by the change of flow rate and mobile phase ratio. Initially, the flow rate was shifted from 2 mL/minute to 1.8 mL/minute and than from 2 mL/minute to 2.2 mL/minute. The passage of the analyte through the system is very

quick at higher flow rate, showing low retention time which results the arising of the smaller peak area. But, the acceptable ranges of the RSD% values at the high flow rate do not exceed the limit. The ratio of the mobile phase from (ACN : Water = 90 : 10) to (ACN : Water = 95 : 05) and from (ACN : Water = 90 : 10) to (ACN : Water = 85 : 15) also showed variable areas under the peaks but still the RSD% value did not cross the standard acceptable ranges.

Depending upon the above results obtained in different parameters against the method developed for the Bifenthrin analyte, it is found that the method is fast, quick, efficient, low cost, repeatable and reproducible with excellent recoveries and is valid equally well for the analysis of Bifenthrin both in the raw material pesticides dosages formulations.

CONCLUSION

A newly developed HPLC-UV method has been proposed for determination of bifenthrin in various insecticides formulations. The developed and validated analytical method depends on the preparation of the analyte samples followed by the HPLC analysis in isocratic elution. The sample preparation step improves the overall performance for the detection of bifenthrin in pesticide formulations form using a single mobile phase. As compared to the analytical methods earlier reported in the literature, this method is simple and more efficient in terms of recovery percentage of the analyte. The validation of the method was checked by the system suitability, linearity, precision, accuracy, repeatability, reproducibility, detection limit, quantification limit, specificity and robustness analysis under various experimental conditions. The Bifenthrin is detected and quantified with high recovery percentage excellent linearity and with low standard deviation values (RSD %). The method developed for the bifenthrin has proved to be accurate, precise, specific and reproducible for the determination of bifenthrin in various formulations. So, the developed analytical method for bifenthrin determination in various formulations of pesticides can be used efficiently at commercial scale.

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