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VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF NOVALURON IN TECHNICAL AND FORMULATION PRODUCTS

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Abstract

A novel method of analysis of Novaluron is validated on reverse phase liquid chromatography using Infinity-1260 series High Performance Liquid Chromatograph (HPLC) of Agilent Technologies equipped with UV-VIS detector and Auto-Sampler. Eclipse C_{18} Column of dimension 250 x 4.6 mm, 5 µm is used. Sample is dissolved in acetonitrile. The composition of mobile phase used is ACN: H_2O (65:35). 1.5 ml min^{-1} is flow rate and detection of Novaluron is carried out on 260 nm and at 8.22 min. Total run time of this chromatography is 10 min. This method is specific, accurate, precise and robust with LOD 7.6 ppm and LOQ 23.1 ppm. All the parameters of validation meet the given criteria. This study has been conducted at Pesticide Quality Control Laboratory Faisalabad as a requirement of ISO/IEC 17025.

Keywords: Method validation, Novaluron, pesticide, High Performance Liquid Chromatography.

INTRODUCTION

Crop yield is function of quality inputs i.e. fertilizers, pesticides and herbicides etc. Weeds and pests affect the crop production negatively. Recently locust problem in Pakistan put the crops in danger and it is a need of the hour that farmer should be provided good quality pesticides. If infested crops are not treated with appropriate pesticides as per recommendation, the yield of the crop drop down drastically. It is observed, especially in cereal crops that the yield reduced by aphid from 10-50 % (PARC, 2014) as well as 12% losses in cotton due the pest attack (NIAB, 2019).

In tropical countries, high temperature and humidity provides conducive environment for the pests to flourish and damage the crops (Zulfiqar et al., 2010; Abhilash and Singh,

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2009; Zulfiqar et al., 2010). Therefore, various pesticide groups are being applied to cope with the pest for crop yield maximization (Rahman and Chima, 2018; Wanwimolruk et al., 2015; Nakano et al., 2004).

Various groups of pesticides are being formulated now-a-days internationally such as carbamates, pyrethroids, nitro compounds, amides organochlorine, heterocyclic pesticides and organophosphates. For plant care and crop yield these pesticides are used frequently and regularly (Mavrikou et al., 2008; Sharifzadeh et al., 2018). Newly discovered bio- pesticides are also in use in the field (Carvalho, 2017).

Aannually about 2.5 million tons of pesticides are being sprayed on the crops globally and incremental trend is noted in the forthcoming times (Pimentel, 1995; FAO, 2002). Pesticide consumption went to 5519 tons from 906 in early eighties and nineties. It is reported that in the last two decades use of pesticides in Pakistan increased and hence the prices of the pesticides also increased steeply (Tariq, 2002). The alarming point noticed that use of pesticides increased about 70 times while the corresponding yields increased only two folds. Khan, 1998 reported that pesticides, herbicides, fungicides, acaricides and fumigants are in use all over the Pakistan in descending order respectively. Novaluron, C17H9ClF8N2O4 1- [3-chloro-4-(1, 1, 2-trifluoro-2-trifluoro-methoxy ethoxy) phenyl]-3-(2, 6-difluorobenzoyl) urea) is a newly introduced pesticide in Punjab for the control of whitefly and leaf miner in cotton, top fruits and vegetables. It is a nitro compound having urea as core part of the compound. It is an insect growth regulator and is considered as an environment friendly pesticide.

To check the quality of a pesticide, a validated analytical method is of prime importance. Lal et al., 2019 emphasized that in natural and synthetic material, the identification and quantification of components is mainly dependent on the analytical methods. They also said that for the quality control, the validation is a key that ensure the suitability of the analytical method for determination. The quality and consistency of results produced by analytical method is related to the outcomes of the method validation. Sophisticated instruments along with the improvement in analytical method, made it possible to use the available resources efficiently and economically (Jay et al., 2003).

Method validation provides surety that the proposed method is suitable for the test (Ermer and Miller, 2006). The method reliability depends on its validation. The results got by a validated method likely to be more authenticated than non-validated method (Wood, 1999). Method validation includes but not limited to limit of quantification and detection, precision, accuracy, linearity and robustness (Thompson et al., 2002; Gul et al., 2015).

MATERIALS AND METHODS

This method is validated for the determination of Novaluron in all pesticide formulations.

Principle

Novaluron is determined at UV wavelength of 260nm through reverse phase high performance liquid chromatography by using acetonitrile as a dissolving solvent.

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Chemicals and Regents

HPLC grade water, Acetonitrile and Certified Reference material (CRM) of Novaluron (99.5% purity) of Sigma Aldrich were used. All the chemicals are of HPLC Grade Quality.

Instrumentation

High performance liquid chromatograph - Agilent 1260 infinity equipped with a constant flow pump, a loop injector, a thermo-stated column oven and a variable wavelength UV detector.

1. Column Agilent Eclipse C_{18} of dimensions; 250 mm x 4.6 mm
2. Ultrasonic bath
3. Filter Assembly
4. Nylon membrane filter of 0.45 µm pore size
5. Analytical Balance
6. Glass Syringe
7. 0.45 µm pore sized nylon syringe filters

Mobile Phase Preparation

Took 65 ml HPLC Grade Acetonitrile and 35 ml HPLC Grade water in a volumetric cylinder and mixed well. Degassed in ultrasonic bath for 5 minutes. Then filtered it through 0.45 µm nylon membrane filter and degas again..

Instrument Conditions (HPLC)

High Performance Liquid Chromatograph (HPLC) conditions are as follow:

Mobiles phase:	65 mL Acetonitrile and 35 mL Water
Column:	C_{18}, 250mm x 4.6 mm, 5 µm
Flow rate:	1.5 mL min^{-1}
Wave length:	260 nm
Column temperature:	40 °C
Injection volume:	5 µL
Retention time:	8.22 min
Duration of analysis:	10.0 min
Diluent:	Acetonitrile
Elution:	Isocratic

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Reference Standard Preparation

Accurately prepare 0.05 % solution of certified reference material (CRM) of Novaluron into a 25 mL volumetric flask using acetonitrile as solvent and place the flask in ultrasonic bath for 1-2 minutes for sonication. Then filter this solution into HPLC vial.

Samples Preparation

Make a solution of 0.05 % of Novaluron from the technical material (97 % purity) into a 25 mL volumetric flask and add acetonitrile up to the mark and place the flask in ultrasonic bath for 1-2 minutes for sonication. Then filter this solution into HPLC vial.

Determination

Active ingredient of Novaluron is determined by using the following equation.

$$\text{Novaluron} = \frac{A \times B}{C \times D} \times E$$

A- Peak area of sample

B- weight of standard

C- Peak area of standard.

D- weight of sample

E- purity of standard

Method validation

Following validation parameters are followed to validate the method.

Specificity

The specificity of the method generally means that a method can separate and identify a single active ingredient from the matrix. (APVMA, 2004). A formulation sample containing the Novaluron is used to determine the specificity of the method.

Accuracy

Accuracy of the method tells about the closeness of the mean of observations to the claimed value. Accuracy of the method is determined by getting repeatability of observations of two analysts. The following formula is used to calculate the accuracy. (Desta and Amare, 2017).

$$\% \text{ Accuracy} = 100 - \% \text{ Error}$$

Precision

Precision is closeness of observations among themselves. Repeatability and intermediate precision are two contributing factors in precision. In repeatability of the method, only one analyst gets 10 reading of a sample and calculates the relative standard deviation at the end. While intermediate precision is calculated against the readings of two analysts (Kalra, 2011).

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Linearity and Range

Linearity and range of the method is determined by making different dilutions of different concentrations. In this case these are 50,100, 200, 300, 400 and 500 ppm.

Limit of Quantification (LOQ)

When linearity is determined, linear equation gives us intercept and slope for calculation the limit of quantification (Jiang et al., 2004; Knoll, 1985).

$$LOQ = 10 \times y - \text{intercept} / \text{slope}$$

Limit of Detection (LOD)

Following formula is used to determine LOD of the method (Jiang et al., 2004; Knoll, 1985).

$$LOD = 3.3 \times y - \text{intercept} / \text{slope}$$

Recovery (%)

Recovery is determined by using the following formula (Wood, 1999; Standard, 2006)

$$\text{Recovery} = \frac{(\text{spiked sample reading} - \text{blank reading})}{\text{Spiked sample reading}} \times 100$$

Robustness

Robustness is the ability of the method to withstand and overcome deliberate changes. (Padmasubashini et al., 2020). In this experiment temperature of oven and flow rate of the mobile phase were changed for checking robustness.

RESULTS AND DISCUSSION

Specificity

With every measurement there is a probability that the observed response is not entirely and specifically caused by the target compound i.e. analyte of interest. If the specificity of the method used, is assessed to be not specific, more certainty concerning the identity of the compound can be achieved by reanalyzing the sample with an alternative analytical procedure (A. Fajgelj et al., 2000). As it is clear from the chromatogram that this method very efficiently separates the active ingredient from the target compound.

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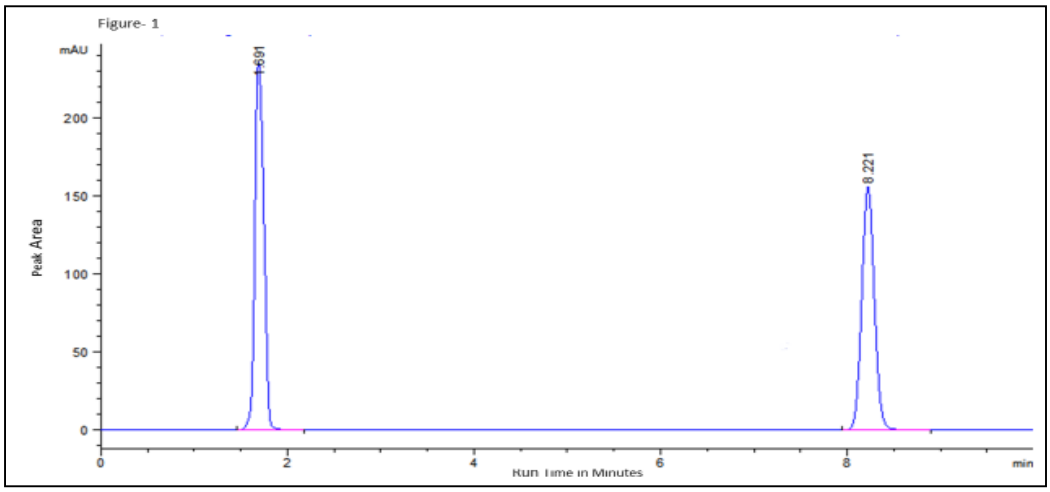

Figure - 1

Accuracy of the Method

The calculated accuracy for this method is 99.28% (Table1). This level of accuracy confirms the reliability of the method to be applied for quality control work. Accuracy criteria set by CIPAC (1999) is >85%. The data met the said level of accuracy.

Table -1

Reading	Analyst 1 (ppm)	Analyst 2 (ppm)	Mean (ppm)
1	498	500	499
2	496	503	499.5
3	495	500	497.5
4	497	496	496.5
5	497	497	497
6	499	501	500
7	497	502	499.5
8	495	499	497
9	498	497	497.5
10	492	501	496.5
Mean X	496.4	499.6	498
Actual Value A	500		
ERROR = (X-A)/A	-0.0072	-0.0008	
% ERROR = ERROR x100	-0.72	-0.08	
% ACCURACY (100-% ERROR)	99.28	99.17	
ACCEPTANCE LIMIT	>85% (CIPAC,1999)		
RESULT (PASS/ FAIL)	PASS		

Precision of the Test Method

Fitness criteria for precision of validated method set by AOAC is < 3% RSD (Latimer, 2019). Data show the level of precision RSD 0.40% (Table 2) and the repeatability RSD 0.27% (Table 3) respectively. This is within the acceptable limit as described by AOAC i.e. 3%. The results of both parameters declare the positive reliability of the test method of Novaluron.

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Table -2

Reading	Analyst 1 (ppm)	Analyst 2 (ppm)	Acceptable Limit	Pass/ Fail
1	498	500	(AOAC) RSD (%) < 3%	Pass
2	496	503		
3	495	500		
4	497	496		
5	497	497		
6	499	501		
7	497	502		
8	495	499		
9	498	497		
10	492	501		
Mean (N=10)	496.4	499.6		
Mean (N=20)	498			
SD of N= 20	2.01108			
%SRD of N= 20	0.4038			

Repeatability

Table - 3

Reading	Analyst 1 (ppm)	Acceptable Limit	Pass/ Fail
1	498	(AOAC) RSD (%) < 3%	Pass
2	496		
3	495		
4	497		
5	497		
6	499		
7	497		
8	495		
9	498		
10	492		
Mean (N=10)	496.4		
SD	1.3642		
%RSD	0.2748		

Linearity and Range of the Test Method

Fig. 2 shows the correlation between the concentration and response (Peak Area) of Novaluron. And it is found linear with the R^2 value of 0.9998 and it is more than the permissive limit i.e. $R^2 > 0.998$ given by CIPAC (CIPAC, 1999).

Table - 4

Sr. No.	ppm	Peak Area
1.	50	575
2.	100	1155
3.	200	2310
4.	300	3465
5.	400	4620
6.	500	5675

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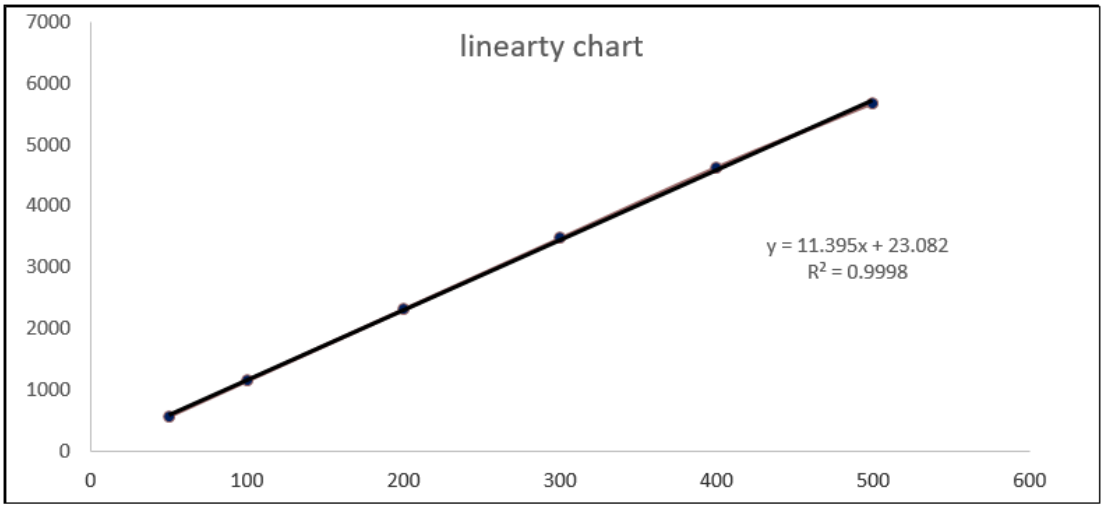
Figure -2



Recovery Percentage of the Method

The recovery percentage values were 99, 97.5, 97, 97.25, and 98.6 against the spiked values of 100, 200, 300, 400, 500 ppm solutions of Novaluron respectively. The data in table 5 confirm the reliability of this method for use to determine the Novaluron from pesticide formulations as well as in quality control system. The given data also meet the criteria for recovery set by CIPAC, 1999 which states that the recoveries should be 70-120 % (Đurović et al., 2016).

Table - 5

Sr. No.	Spiking Level (A)	Experimental Value (X)	Recovery (%)	Acceptable Criteria	Pass/ Fail
1	100	99	99.0	70-120% (AOAC,1999)	PASS
2	200	195	97.5		
3	300	291	97.0		
4	400	389	97.25		
5	500	493	98.6		

Limit of Detection and Limit of Quantification of Method

The limit of detection is the minimum amount of the solute that detector detects through given wavelength in the method. On the other hand, limit of quantification is the minimum concentration that can be quantified accurately (Jiang et al., 2004; Knoll., 1985). The limit of detection and limit of quantification for this method is calculated using linear equation are 7.6 and 23.1 ppm respectively.

Robustness of The Method

Although deliberate changes create some system changes like pressure of the system and shifting of retention time but there is no change in the recovery percentage and the % RSD of the trial as depicted by the data given in the table 6 and figure 3. Both the recovery % age and % RSD meet the precision and the recovery criteria that affirms that

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this very method is robust in nature and can be used for any purposes anywhere for the said pesticide formulation (chemistry).

Table - 6

Parameter	Condition	% RSD	Recovery %
Column temperature	30˚C	0.25	99.72
	35˚C	0.90	99.13
Flow rate	1 ml/min	0.10	99.84
	1.5 ml/min	0.20	99.10

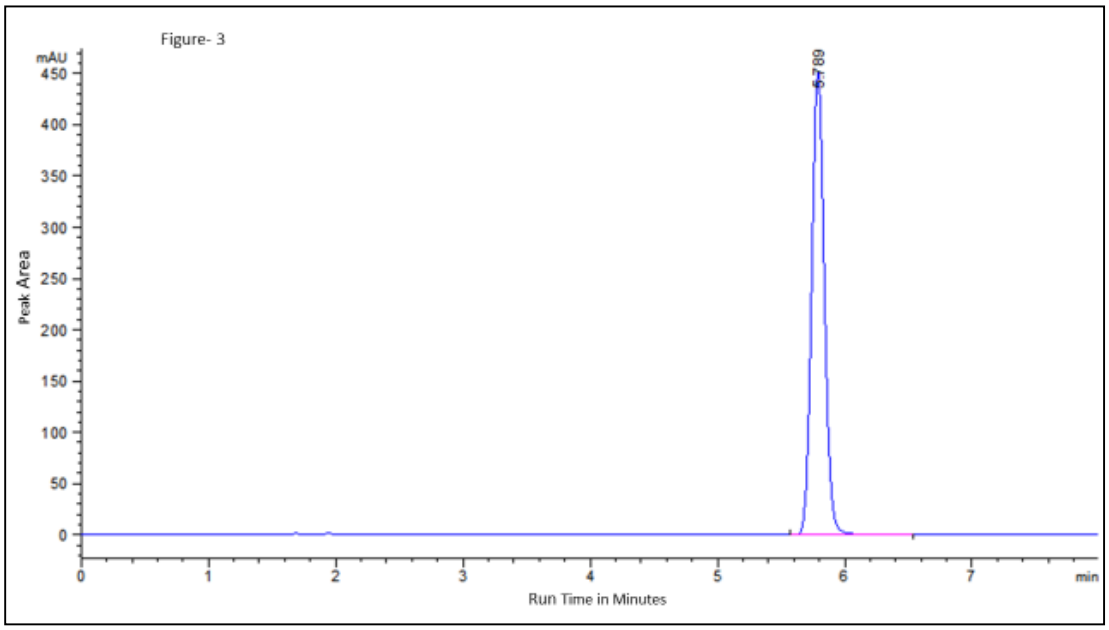

Figure -3

CONCLUSION

Method validation is a cumbersome and time consuming process. Keeping in view the resources this method is validated. This method fulfill all the needs required for quality control work. This method is precise, accurate and robust and simple to use.

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Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
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