Development and Validation of Novel HPLC Method for Determination of Nitenpyram Insecticide in Commercial Samples

Ahmed Yar[1], Tariq Mahmood Ansari[1], Asad Raza[2], Zeenat Javeed[3], Muhammad Asif[4]

[1]Institute of Chemical Sciences, Bahauddin Zakariya University Multan, 60800, Pakistan

[2]Department of Chemistry, Govt. College Civil Lines Multan, Punjab, Pakistan

[3]Soil and Water Testing Laboratory, Bahawalpur, Punjab, Pakistan

[4]Soil and Water Testing Laboratory, Bahawalnagar, Punjab, Pakistan

Corresponding Author email address: ahmed_gagran@yahoo.com

Abstract— In this study, a novel, rapid, economical, accurate, reproducible and sensitive analytical method for the determination of Nitenpyram using reverse-phase high-performance liquid chromatography with UV-Visible detector has been reported. This method is equally useful and valid for the detection and quantification of Nitenpyram in different dosage formulations and raw material forms with outstanding recoveries up to 100%. The method has been validated with superb linearity value R^2 = 0.9999. LOD was measured as 0.51 mg/L while the LOQ was 1.69 mg/L. The analytical method has shown high precision (RSD ≅ 0.17%) while the accuracy measured in terms of recovery percentage was approximately 100% under optimized experimental conditions. Finally, the robustness results of the proposed method by altering the flow rate and the mobile phase concentration ratios were within the permissible accepted values in terms of Relative Standard Deviation (RSD ≤ 2%). So, the proposed method can be used in quality control laboratories of the pesticide industry at a commercial scale even for the determination of Nitenpyram in samples such as Soluble Liquid (SL), Wettable Powder (WP), Water Dispersible Granules (WDG), Emulsifiable Concentrate (EC) and Technical (Pure Active Ingredient), etc.

Keywords: HPLC-UV, Nitenpyram, Insecticide, Method Validation

1 INTRODUCTION

Pesticides are the chemicals which are used to kill the pests. Pests are those insects which harm our crop plants. So, the pesticides include a wide variety of classes like the insecticides, avicides, fungicides, bactericides, viricides, herbicide and miticides.

History of nitenpyram was reported by (Minamida et al., 1993). Nitenpyram was under investigation for crop protection. Nitenpyram is the common name in the insecticides. IUPAC name of nitenpyram is (*E*)-*N*-(6-chloro-3-pyridylmethyl)-*N*-ethyl-*N*'-methyl -2-nitrovinylidenediamine and Chemical Abstracts name is *N*-[(6-chloro-3-pyridinyl) methyl]-*N*-ethyl-*N*'-methyl-2-nitro-1, 1-ethenediamine respectively. Fig.1 shows the structural formula of Nitenpyram.

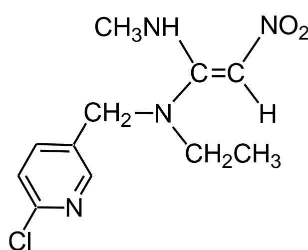

Fig.1. Structural Formula of Nitenpyram Insecticide

Nitenpyram is an important and effective type of insecticide belonging to the chemical family of Neonicotinoid pesticides widely and efficiently used against the sucking and biting insects. Since the launching of pyrethroids, the neonicotinoid is the fast growing class of insecticides.

Nitenpyram is an agonist of the nicotinic acetylcholine receptor, affecting cholinergic transmissions in the insect central nervous system. Mode of action of the Nitenpyram is the systemic insecticide with translaminar activity with contact and stomach action. Nitenpyram is efficiently used for the control of aphids, thrips, leafhoppers, whitefly and other sucking insects on rice and glasshouse crops. On rice, applied at 15-75 g/ha (foliar), 75-100 g/ha (dust) or 300-400 g/ha (soil treatment). Nitenpyram is also used for the control of fleas on cats and dogs. Types of nitenpyram formulation available are DP (Dustless Powder), GR (Granules) and SP (Soluble Powder). Selected products are Best Guard (Sumitomo Chemical Takeda) and other products are Capstar (flea control) (Sumitomo Chemical Takeda, Novartis A H),

Programa (flea control, Japan) (Sumitomo Chemical Takeda, Novartis A H), Takestar (flea control, Japan) (Sumitomo Chemical Takeda, Novartis (Tomlin, 2009). By studying the resistance in different insect species against the nitenpyram, a study was performed on 09 different ordinarily used neonicotinoids; Nitenpyram has shown the greatest increase in the resistance of the group in brown plant hoppers which was a common pest in 2011 – 2012.It had been observed in the study of the effects of Nitenpyram on aquatic animals, sixty days chronic toxicity test was done on rare middows generally on the fish model in China (Pisa et al., 2021). Nitenpyram has shown no adverse effects on the immune system of the aquatic animals. The similar study of Nitenpyram showed an adverse effect by affecting the enzymes that were inhibiting the synthesis of reactive oxygen species has damaged the oxidative DNA with chronic exposure on the DNA of zebra fish (Hong et al., 2018). In mammals the study of adverse effects was done in Oxford University on rats. An LD_{50} test was performed by selecting the dosages 1680 mg and 1575 mg per kg body weight on both male and female rats respectively. The Nitenpyram is seemed to be safe for the humans and animals because of the over dosage of the compound reaches to the grams.

By studying the degradation of the nitenpyram in different types of the water, an interesting thing was observed (Yan et al., 2015). Nitenpyram was showing degradation in the drinking water in the form of hydrolysis of the compound found by the researchers when they were testing degradation of nitenpyram in ground water, surface water and finished drinking water. Nitenpyram has also shown degradation by UV light and sunlight exposure.

A wide variety of literature is available for nitenpyram residual analysis and many methods were developed to check nitenpyram residues in fruits, vegetables, oils, waste water, in blood plasma, foods by using various analytical techniques especially the chromatographic, capillary electrophoresis, electro kinetic chromatography, carbon electrode and photochemistry techniques. HPLC-MS/MS and UHPLC determination of Neonicotinoid residues in fruits, vegetables, human urine and in singing bird plasma (Chen et al., 2013; Garrido Frenich et al., 2008; Ge et al., 2020; Hao et al., 2018; Iwafune et al., 2014; Jovanov et al., 2013; Liu et al., 2010; López-García et al., 2017; Nelson et al., 2015; Nguyen et al., 2009; Noestheden et al., 2016; Sack et al.; Saito-Shida et al., 2014; Valverde et al., 2018; Xiao et al., 2011; Yoshida et al., 2013; Zhang et al., 2018). HPLC DAD, Mass spectrometry and electro kinetic chromatography analysis of Neonicotinoid residues in honey bees, vegetables and fruits (Akram, 2015; Campillo et al., 2013; Farouk et al., 2016; Obana et al., 2002; Wang et al., 2019; Watanabe et al., 2016; Yang et al., 2010; Zhang et al., 2012). Neonicotinoid determination by Bismuth Modified Carbon Electrode, by ELISA and Photo chemistry absorption spectra (Ezell et al., 2019; Guzsvány et al., 2011; Watanabe et al.,2018; Zhang et al., 2017). Degradation of Nitenpyram in wastewater by Gas Diffusion Electrode (Li et al., 2013). A new technique of CE-MS has also been introduced for Neonicotinoid insecticide determination (Sánchez-Hernández et al., 2014). All of these methods are specifically designed for certain applications for extraction and determination of Nitenpyram residues in fruits, vegetables, wastewater, drinking water, honey bee, fruits, plasma but none of the HPLC-UV method has still been developed which is simple, economical and easily handled with minimum retention time for the quantitative and qualitative determination of Nitenpyram insecticide in quality control laboratory either for raw material and/or for Nitenpyram insecticide dosages formulation at commercial and/or industrial scale.

It is worth mentioning that the developed and under-developed countries have agrochemical industries which are the backbone of their economy. All of these industries have high performance liquid chromatographic instruments with UV-Visible detectors in their quality control laboratories which are most commonly used and applied for the determination of pesticides. These instruments are economical compared to HPL-DAD and other equivalents in liquid chromatography. In addition, none of official methods for determination of nitenpyram contents has been reported in CIPAC (Collaborative International Pesticide Analytical Council), FAOs (Food & agricultural Organization) and AOAC methods for pure, raw material and pesticides dosage formulations.

Keeping in view, the extensive and widespread use of nitenpyram insecticide in agro sector against the variety of plant diseases, this study was aimed to develop and validate an analytical method employing HPLC–UV technique for the determination of the nitenpyram contents in pure, raw material and pesticides dosage formulations.

2 EXPERIMENTAL

2.1 Reagents and Chemicals

Analytical Reagent Grade chemicals were used during the whole experimental work. Acetonitrile and methanol of HPLC gradient grade from Duksan Pure Chemicals Korea, Water HPLC Grade from VWR Chemicals and Nitenpyram Analytical Standard of Known Purity 99.4% from Chem Services, USA was obtained. A sample of Nitenpyram 10% SL (soluble liquid) product marketed by name of Jasper was collected from Solex Chemicals Quality Control Laboratory Multan, Pakistan. The Samples of 50% WG (Wettable Granules) and WP (Wettable Powder) were purchased from the local market of Multan, Pakistan.

2.2 Apparatus

A filtration assembly (Glasco) with vacuum pump was used for mobile phase filtration. Filter paper of 0.25µm and 0.45µm (Sartorius) were used for filtration of mobile phase. Ashless filter paper (#42) from Sartorius was used for filtration of sample solution. Weighing of the sample and standard was performed using a highly sensitive analytical weighing balance (Mettler Toledo) model AB204-S. An ultrasonic water bath (GT Sonic D3, China) was used for the extraction of the sample and standard solutions. Certified glassware from Iwaki Pyrex was used during the whole experimental and practical work.

HPLC analysis of nitenpyram was performed with Shimadzu Japan HPLC system consisting of LC-20AT pump and SPD-20A UV-VIS detector. An HPLC system of 10 AT of SPD -10A VP UV-VIS detector was also used during the experimental work. A zorbax 250mm x 4.6mm (i.d) packed C18 column with 5µm (partical size) from Agilent Technologies was set at normal room temperature. Isocratic elution was performed for the separation of nitenpyram contents by using the mobile phase (Methanol 30% + Acetonitrile 30% + Water 40%). The flow rate used during the analysis was 1 mL/min. The analyte volume injected was 20 µl. The micro glass syringe with stainless steel piston of 50 µl was arranged from SGE. The wavelength used for the detection of nitenpyram was set at 254 nm. The content (%age) of the nitenpyram sample solution was determined by comparison of peak area of the analyte peak with the peak area of analytical standard solution peak. The nitenpyram contents peak was detected at the retention time of 3.7.

2.3 Preparation of Analytical Standard Solution

10 mg of nitenpyram pure analytical standard solution was prepared from 99.5% pure analytical standard with the accuracy of ± 0.01 mg into a separate 50 mL volumetric flask. The analytical standard of nitenpyram was dissolved into the 10 mL of diluent (Methanol 30% + Acetonitrile 30% + Water 40%) by sonicating moderately and then this analytical standard solution was cooled to room temperature at room environment and the volume was made up to 50 mL with diluent. The analytical standard solution was then shaken vigorously to homogenize dissolution. This solution of analytical standard was found to be stable for 24 hours. Working standards of 0, 25, 50, 75, 100 and 125 mg of nitenpyram from pure analytical standard (99.5%) for linearity curve were prepared by diluting up to the mark (50 mL) with diluent (Methanol 30% + Acetonitrile 30% + Water 40%). All the working standard solutions were filtered with membrane filter paper (0.45µm) before injecting. These working standard solutions were then analyzed on HPLC, the data was recorded on the chromatograms and the percentage recovery was calculated. The whole process was repeated three times.

2.4 Preparation of the 10% Nitenpyram (Jasper) Product Sample Solution

About 10 mg ± 0.01 mg of pure nitenpyram contents sample solution was prepared on 100% purity basis from Jasper 10% SL (Nitenpyram 10% SL) in 50 mL volumetric flask. The volume was made up with diluent (Methanol 30% + Acetonitrile 30% + Water 40%). The product sample solution was shaken vigorously for homogeneity. The sample solution was filtered with membrane filter paper (0.45µm) and was maintained to room temperature for analysis on HPLC. The data was recorded on chromatograms. The percentage recovery was calculated by repeating the whole process three times which has been shown in results and discussion.

2.5 HPLC Conditions and Method Optimization

Different Chromatographic parameters were set by changing the various mobile phase compositions, rate of flow and detector wavelength. By varying the ratios of HPLC gradient grade solvents for example Acetonitrile and Methanol were set with water (Methanol 30% + Acetonitrile 30% + Water 40% to 100:0:0 at interval of 10). This practice was done for the mobile phase optimization to obtain best separation of the analyte with good resolution. The flow rate of the mobile phase was changed between 0.5mL/min to 1mL/min at changing interval of 0.1mL/min. During the whole analysis process, isocratic elution of mobile phase was followed. Degassing of mobile phase was done by ultrasonic water bath after passing through 0.45µm nylon membrane filter paper using vacuum pump filtration system. The process of the separation of analyte was done by using C-18 column at the room temperature. Various wavelengths of UV range between 200 to 300nm at the interval of 10 nm were tested to decide λ_{max} and optimum chromatographic responses to minimize interferences received from inert materials available in the formulated products (Jovanov et al., 2013). The optimum flow rate and wavelength were changed deliberately to perform the robustness test. Comparison of the results achieved by changing each parameter was checked accordingly.

2.6 Proposed Method

HPLC-UV system (LC-20AT with SPD-20A detector) used was from Shimadzu Japan where detector wavelength used was 254 nm and Column C18 Zorbax Agilent Technologies serial number 560562 (250mm x 4.6mm (i.d) x 5 µm). The mobile phase used was **(**Methanol 30% + Acetonitrile 30% + Water 40%). Flow rate was maintainedat1.0 mL/min and the approximate retention time was found to be 3.7.

The nitenpyram contents were calculated by using the following equation "Eq. (1)".

$$Nitenpyram\ contents\ \%\ \left(\frac{w}{w}\right) X1 = {A_2\ x\ m_1\ x\ P}/{A_1\ x\ m_2} \quad (1)$$

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

Where

A_1 = Average peak area of the Nitenpyram in the standard solution
A_2 = Average peak area of the Nitenpyram in the sample solution
m_1 = mass of Nitenpyram standard (mg)
m_2 = mass of Nitenpyram sample (mg)
P = Purity of Nitenpyram analytical standard
Graphical scheme of experimental work has shown in Fig. 2.

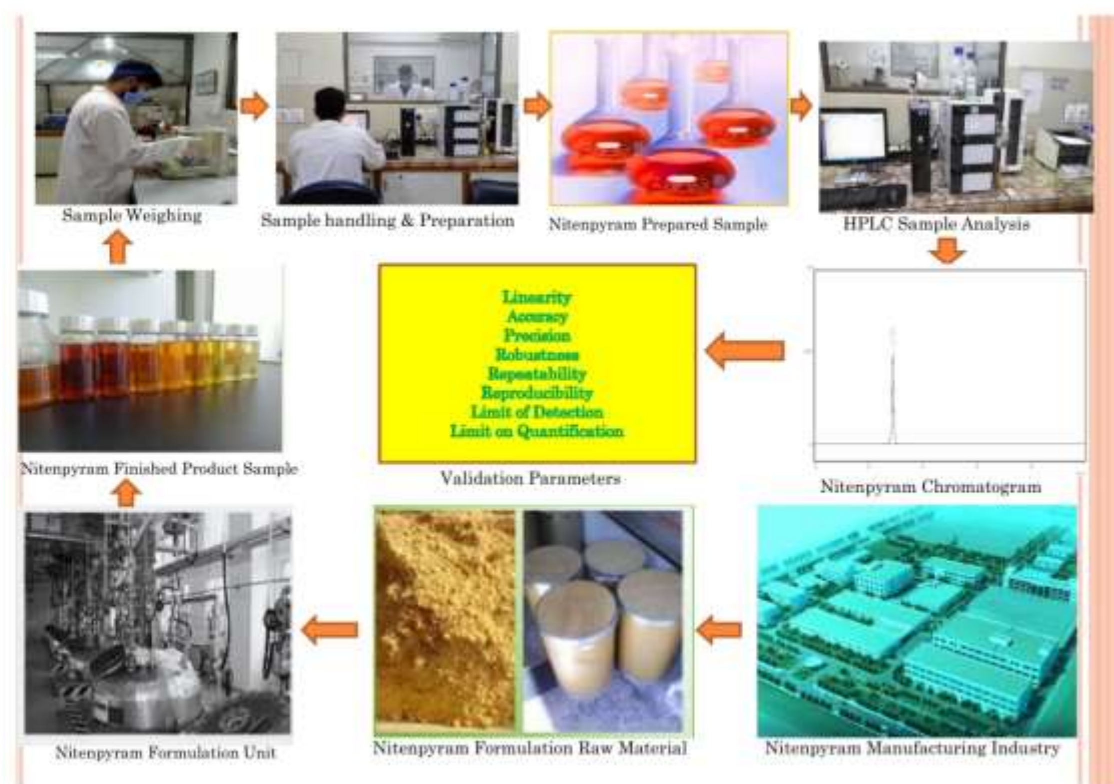

Fig. 2. Graphical scheme of the experimental work

3 Results and Discussion

In this work, various parameters have been optimized for valid analytical measurement of Nitenpyram in pure, raw material and pesticide dosage formulations using HPLC-UV technique. Details have been given in the following sections:

3.1 Method Validation

The HPLC chromatograms of nitenpyram in standard as well as in sample solutions (Figures 3a and 3b) have shown the same retention times (3.7 min.).

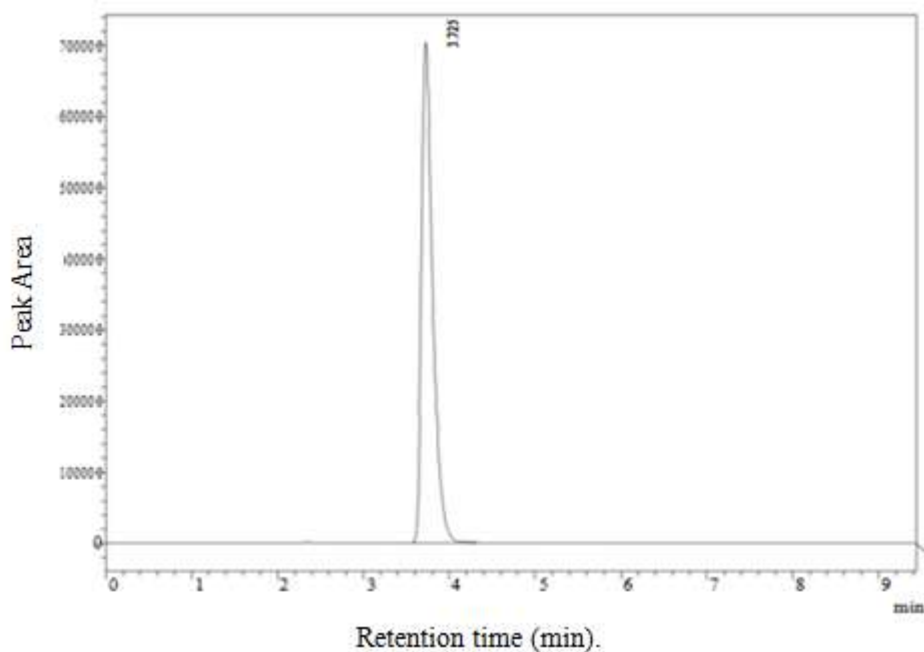

Fig. 3 (a) HPLC chromatogram of the Nitenpyram standard solution

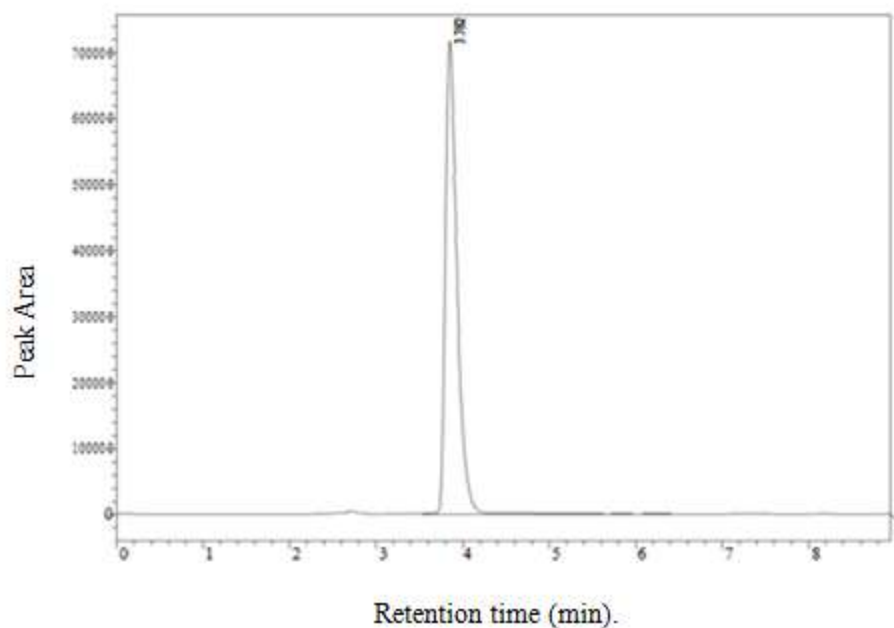

Fig. 3 (b) HPLC Chromatogram of the Nitenpyram sample solution

3.2 Linearity for Nitenpyram

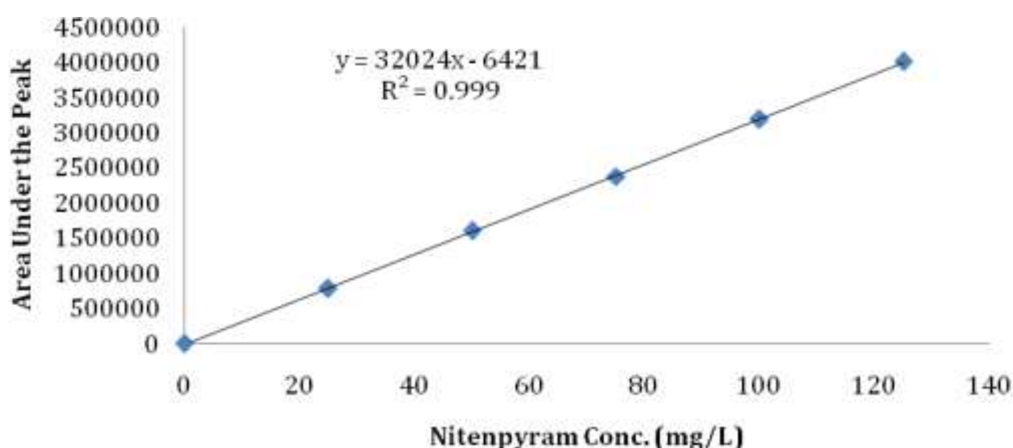

Fig. 4 Calibration curve of Nitenpyram by the proposed method

Fig.4 shows the linearity curve which has been plotted between the nitenpyram concentration and the peak areas. The Linearity of the method developed for the Nitenpyram which was evaluated by using different concentrations of 25 mg, 50 mg, 75 mg, 100 mg and 125 mg per 50 mL of Nitenpyram pure active ingredient from analytical standard of 99.5%. The value of correlation coefficient (R^2) in straight line obtained was R^2 = 0.9999. The R^2 value shows that extraction/solubility is verified by this HPLC method for Nitenpyram pure active ingredient contents.

3.3 Precision for Nitenpyram

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

Area of Standards Nitenpyram	
1	6372997
2	6358237
3	6364451
4	6355210
5	6382052
Average	6366589
Standard Deviation	± 10999
RSD%	0.17%

For the system suitability criteria, the value of relative standard deviation (RSD = ± 2%) for nitenpyram was obtained 0.17% by the five replicate readings (Table 1).

3.4 Selectivity/Specificity for Nitenpyram

Table 2: Specificity of the developed method for Nitenpyram

Product	Results in Mixture	Mean Result in Soul Sample		Recovery (80% – 120%)	Remarks
		Area under the peak of the standard solution	Area under the peak of the sample solution		
Nitenpyram	10.00%	6366589	6648733	100.18%	Pass
		10.01%			

The method is specified and selective for nitenpyram 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).

3.5 Accuracy for Nitenpyram

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: Accuracy of the developed method for determination of Nitenpyram

Known Conc. of Nitenpyram (mg/L)	Mean (Peak Area) of Nitenpyram Standard solution	Mean (Peak area) of Nitenpyram Sample solution	Measured Conc. of Nitenpyram in sample solution (mg/L)	Percentage Recovery of Nitenpyram (%)
25	790611	789515	24.97	99.88%
50	1602606	1590964	49.64	99.28%
75	2379800	2374775	74.84	99.79%
100	3185920	3175859	99.68	99.68%
125	4011610	4001044	124.67	99.74%

Percentage recoveries of nitenpyram in sample solutions range between 99.28 to 99.88% indicating that the developed method is accurate.

3.6 Repeatability for Nitenpyram

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

Sr. #.	Observation	Nitenpyram (Peak Area)
1	Reading 1	6646421
2	Reading 2	6661072
3	Reading 3	6642080
4	Reading 4	6629107
5	Reading 5	6664987
6	**Mean**	6648733

7	**SD**	14588
8	**RSD%**	0.22%

In evaluating the repeatability parameter (Table 4) for the nitenpyram developed method it has been observed that by analyzing the Nitenpyram analyte within different interval of times upon same conditions and instruments, the results showed the RSD% do not deviate the standard value (RSD% ≤ 2%).

3.7 Reproducibility for Nitenpyram

Table 5: Reproducibility of the developed method for Nitenpyram

Sr.#	Observation	Nitenpyram (Peak Area)	
		HPLC – 20AT	HPLC – 10AT
1	Reading 1	6646421	5615444
2	Reading 2	6661072	5584652
3	Reading 3	6642080	5609485
4	Reading 4	6629107	5620519
5	Reading 5	6664987	5620700
6	**Mean**	6648733	5610160
7	**S.D**	14588	14979
8	**RSD (%)**	0.22%	0.27%

Table 6: Reproducibility of the developed method for determination of Nitenpyram

Formulation	Company	Proposed Method	
		Recovery %age	RSD %
Nitenpyram 10% (SL)	Industry A	100.44%	0.41%
Nitenpyram 10% (SL)	Industry B	100.74%	0.35%
Nitenpyram 10% (SL)	Industry C	100.98%	0.38%
Nitenpyram 50%(WG)	Industry D	101.08%	0.27%
Nitenpyram 10% (SL)	Industry E	100.24%	0.41%

Table 7: Inter Laboratory Comparison Test for multiple Nitenpyram pesticide formulations

Sr.#	Laboratory	Formulation Type			
		Nitenpyram 10% SL		Nitenpyram Tech 95%	
		*Results	%RSD	*Results	%RSD
01	**Lab 01**	10.01%	0.24%	94.99%	0.10%
02	**Lab 02**	9.98%	0.11%	95.03%	0.11%
03	**Lab 03**	10.04%	0.35%	95.05%	0.14%
04	**Lab 04**	10.02%	0.18%	95.01%	0.09%

05	Lab 05	10.01%	0.31%	95.04%	0.13%

(*) Average of 5 replicates

While performing the reproducibility parameter on two HPLC instruments naming HPLC -20AT with SPD–20A detector and HPLC -10AT with SPD–10A from Shimadzu corporation Japan, (Table 5) it had been observed that the developed method for the nitenpyram analyte did not deviate the standard value of (RSD% ≤ 2%) while performing the same nitenpyram analyte on another instrument HPLC LC-10AT with SPD-10AVP UV-Visible detector. Hence, the developed analytical method found fit for analyzing nitenpyram contents equally well in the pure, raw material and pesticide dosages formulations in quality control laboratory.

Table 6 shows that the method is reproducible and selective when applied on different formulations during different intervals of time .The recovery percentage and relative standard deviation are always remain within the declared specified limits.

Table 7 depicts the Inter Lab Comparison (ILC) Results of nitenpyram which again shows a great harmony in results in terms of standard prescribed limits.

3.8 Limit of Detection and Limit of Quantification for Nitenpyram

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

No. of Readings	Nitenpyram (mg/L)
1	49.88
2	50.04
3	49.73
4	49.99
5	49.88
Mean	49.90
SD (So)	0.1197
Śo=SQR(2)* so	0.17
LOD=3* Śo	**0.51**
LOQ=10* Śo	**1.69**

Table 8 shows that the value of LOD for nitenpyram was found to be 0.51 mg and that the value of LOQ was found to be 1.69 mg which is the clear indication of signal-to-noise ratio 3:1 for LOD and LOQ.

3.9 Robustness for Nitenpyram

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

Sample No.	Change of Flow Rate			Change of Mobile Phase		
	Peak area at 0.8mL/min	Peak area at 1.0mL/min	Peak area at 1.2mL/min	ACN : Methanol : Water 35 : 35 : 30	ACN : Methanol : Water 30 : 30 : 40	ACN : Methanol : Water 25 : 25 : 50
1	8468495	6646421	5899164	5412847	6646421	9652507
2	8442435	6661072	5877218	5395217	6661072	9650201
3	8456063	6642080	5864185	5388040	6642080	9674288
4	8464520	6629107	5836364	5411653	6629107	9662556
5	8434076	6664987	5906784	5366598	6664987	9691371
Mean	8453118	6648733	5876743	5394871	6648733	9666185

Std. Deviation	14593	14588	28256	19045	14588	16998
RSD %	0.17%	0.22%	0.48%	0.35%	0.22%	0.18%

While performing robustness (Table 9) of the method developed for the nitenpyram it had been observed that by increasing the flow rate of mobile phase from 1.0 mL/min to 1.2 mL/min the area under the peak became decreasing. While the RSD% was remained within the prescribed limits. (RSD% ≤ 2%) While decreasing the flow rate of mobile phase from 1.0 mL/min to 0.8 mL/min the area under the peak became increasing. In this case again the RSD% did not deviate from the standard value (RSD% ≤ 2%). Similarly robustness of the method had been evaluated by changing the mobile phase concentrations from (ACN : Methanol : Water = 30 : 30 : 40) to (ACN : Methanol : Water = 25 : 25 : 50) the area under the peak became increasing but the RSD% do not deviate the prescribed standard value (RSD% ≤ 2%). During the decrease of water ratio in the mobile phase (ACN : Methanol : Water = 30 : 30 : 40) to (ACN : Methanol : Water = 35 : 35 : 30) the area under the peak became decreasing but again the RSD% shows no deviation from the standard value (RSD% ≤ 2%). So, it has been proved that the developed analytical method for nitenpyram contents measurement is found to be robust & rigid and regret the minimal changes in the mobile phase concentration and/or in flow.

3.10 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 Nitenpyram

Validation Parameter	Results (Nitenpyram)		Acceptance Criteria
Linearity	Correlation Coefficient = 0.9999		Correlation Coefficient NLT* 0.97
Precision	0.17% RSD		% RSD NMT* 2.0
Accuracy	**Concentration (mg/L)**	**% Recovered**	% Recovery within 80% - 120%
	25	99.86%	
	50	99.27%	
	75	99.79%	
	100	99.68%	
	125	99.74%	
Repeatability	0.22% RSD		RSD ≤ 2.0%
Reproducibility	**HPLC – 20AT**	**HPLC – 10AT**	
	0.22% RSD	0.27% RSD	
Detection and Quantitation Limit	**LOD**	**LOQ**	-
	0.51 mg/L	1.69 mg/L	
Robustness	**Change**	**% RSD**	% RSD NMT 1.5
	(Flow rate) 0.8 mL	0.17%	
	(Flow rate) 1.0 mL	0.22%	
	(Flow rate) 1.2 mL	0.48%	
	(Mobile Phase) Methanol :ACN : Water 350 : 350 : 300	0.35%	
	Methanol :ACN : Water 300 : 300 : 400	0.22%	

	Methanol :ACN : Water 250 : 250 : 500	0.18%

* 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)

Table 10 shows the summary of the parameters of the developed method for nitenpyram. 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 nitenpyram contents by HPLC both in raw material and pesticide dosage formulations in quality control sector of the industries. Analytical standard solution for the nitenpyram 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.

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 nitenpyram 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 (99.88%) while at 50 mg (99.28%), 75 mg (99.79%), 100 mg (99.68%) and 125 mg (99.74%). So, the proposed method for the nitenpyram 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 nitenpyram 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 1 mL/min to 0.8 mL/min and than from 1 mL/min to 1.2 mL/min. 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 (Saleh et al., 2021). The ratio of the mobile phase from (ACN : Methanol : Water = 30 : 30 : 40) to (ACN : Methanol : Water = 35 :35 : 30) and from (ACN : Methanol : Water = 30 : 30 : 40) to (ACN : Methanol : Water = 25 : 25 : 50) 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 nitenpyram 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 nitenpyram both in the raw material pesticides dosages formulations.

4 CONCLUSION

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

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REFERENCES

1. Akram, S. (2015). Determination of Neonicotinoids in selected fruits and vegetables and their impact on nutritional attributes. University of Agriculture, Faisalabad.

2. Campillo, N., Viñas, P., Férez-Melgarejo, G., & Hernández-Córdoba, M. (2013). Liquid chromatography with diode array detection and tandem mass spectrometry for the determination of neonicotinoid insecticides in honey samples using dispersive liquid–liquid microextraction. *Journal of Agricultural Food Chemistry,* 61, 4799-4805.

3. Chen, M., Collins, E.M., Tao, L., & Lu, C. (2013). Simultaneous determination of residues in pollen and high-fructose corn syrup from eight neonicotinoid insecticides by liquid chromatography–tandem mass spectrometry. *Anaytical and Bioanalytical Chemistry,* 405, 9251-9264.

4. Ezell, M.J., Wang, W., Shemesh, D., Ni, A., Gerber, R.B., & Finlayson-Pitts, B.J. (2019). Experimental and theoretical studies of the environmental sensitivity of the absorption spectra and photochemistry of nitenpyram and analogs. *ACS Earth and Space Chemistry*, 3, 2063-2075.

5. Farouk, M., Hussein, L.A.E.A., & El Azab, N.F. (2016). Simultaneous determination of three neonicotinoid insecticide residues and their metabolite in cucumbers and soil by QuEChERS clean up and liquid chromatography with diode-array detection. *Analytical Methods*, 8, 4563-4575.

6. Garrido Frenich, A., Martínez Vidal, J., Pastor-Montoro, E., & Romero-González, R. (2008). High-throughput determination of pesticide residues in food commodities by use of ultra-performance liquid chromatography–tandem mass spectrometry. *Anaytical and Bioanalytical Chemistry*, 390, 947-959.

7. Ge, S., Wang, Y., Song, Q., Chen, L., Zhang, Y., & Hu, D. (2020). Determination of nitenpyram dissipation and residue in kiwifruit by LC-MS/MS. *Food Additives & Contaminants*, 37, 955-962.

8. Guideline, I.H.T. (2022). Analytical Procedure Development Q14. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Geneva, Switzerland.

9. Guzsvány, V., Papp, Z., Zbiljić, J., Vajdle, O., & Rodić, M. (2011). Bismuth modified carbon-based electrodes for the determination of selected neonicotinoid insecticides. *Molecules*, 16, 4451-4466.

10. Hajare, A.A., Powar, T.A., Bhatia, N.M., & More, H.N. (2016). Development and validation of RP-HPLC method for determination of doxorubicin hydrochloride from vacuum foam dried formulation. *Research Journal of Pharmacy and Technology,* 9, 1352-1356.

11. Hao, C., Eng, M.L., Sun, F., & Morrissey, C.A. (2018). Part-per-trillion LC-MS/MS determination of neonicotinoids in small volumes of songbird plasma. *Science of the Total Environment,* 644, 1080-1087.

12. Hong, X., Zhao, X., Tian, X., Li, J., & Zha, J. (2018). Changes of hematological and biochemical parameters revealed genotoxicity and immunotoxicity of neonicotinoids on Chinese rare minnows (Gobiocypris rarus). *Environmental Pollution,* 233, 862-871.

13. Iwafune, T., Ogino, T., & Watanabe, E. (2014). Water-based extraction and liquid chromatography–tandem mass spectrometry analysis of neonicotinoid insecticides and their metabolites in green pepper/tomato samples. *Journal of Agricultural Food Chemistry,* 62, 2790-2796.

14. Jovanov, P., Guzsvány, V., Franko, M., Lazić, S., Sakač, M., Šarić, B., & Banjac, V. (2013). Multi-residue method for determination of selected neonicotinoid insecticides in honey using optimized dispersive liquid–liquid microextraction combined with liquid chromatography-tandem mass spectrometry. *Talanta*, 111, 125-133.

15. Li, S.P., Ma, X.R., Cao, X.H., & Dong, Y.W. (2013). Electro-catalytic degradation of nitenpyram wastewater using C/PTFE gas diffusion electrode, Advanced Materials Research. *Trans Tech Publications*, pp. 747-752.

16. Liu, S., Zheng, Z., Wei, F., Ren, Y., Gui, W., Wu, H., & Zhu, G. (2010). Simultaneous determination of seven neonicotinoid pesticide residues in food by ultraperformance liquid chromatography tandem mass spectrometry. *Journal of Agricultural Food Chemistry,* 58, 3271-3278.

17. López-García, M., Romero-González, R., Lacasaña, M., & Frenich, A.G. (2017). Semiautomated determination of neonicotinoids and characteristic metabolite in urine samples using TurboFlow™ coupled to ultra high performance liquid chromatography coupled to Orbitrap analyzer. *Journal of Pharmaceutical and Biomedical Analysis*, 146, 378-386.

18. Minamida, I., Iwanaga, K., Tabuchi, T., Aoki, I., Fusaka, T., Ishizuka, H., & Okauchi, T. (1993). Studies on acyclic nitroethene compounds. Part 2. Synthesis and insecticidal activity of acyclic nitroethene compounds containing a heteroarylmethylamino group. *Journal of Pesticide Science,* 18, 41-48.

19. Nelson, M.A., Bedner, M., Lang, B.E., Toman, B., & Lippa, K.A. (2015). Metrological approaches to organic chemical purity: primary reference materials for vitamin D metabolites. *Anaytical and Bioanalytical Chemistry,* 407, 8557-8569.

20. Nguyen, T.D., Yun, M.Y., & Lee, G.-H. (2009). A multiresidue method for the determination of 118 pesticides in vegetable juice by gas chromatography− mass spectrometry and liquid chromatography− tandem mass spectrometry. *Journal of Agricultural Food Chemistry,* 57, 10095-10101.

21. Noestheden, M., Roberts, S., & Hao, C. (2016). Nitenpyram degradation in finished drinking water. *Rapid Communications in Mass Spectrometry,* 30, 1653-1661.

22. Obana, H., Okihashi, M., Akutsu, K., Kitagawa, Y., & Hori, S. (2002). Determination of acetamiprid, imidacloprid, and nitenpyram residues in vegetables and fruits by high-performance liquid chromatography with diode-array detection. *Journal of Agricultural Food Chemistry,* 50, 4464-4467.

23. Pisa, L., Goulson, D., Yang, E.-C., Gibbons, D., Sánchez-Bayo, F., Mitchell, E., Aebi, A., van der Sluijs, J., MacQuarrie, C.J., & Giorio, C. (2021). An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems. *Environmental Science and Pollution Research,* 28, 11749-11797.

24. Sack, C., Michael Smoker, K., MacMahon, S., Masse, C., Michael, N., Mercer, G., Neuhaus, B., Cassias, I., Chang, E., Collaboration of the QuEChERS Procedure for the Multiresidue Determination of Pesticides by LC-MS/MS In Raw Agricultural Commodities.

25. Saito-Shida, S., Nemoto, S., & Matsuda, R. (2014). Multiresidue analysis of pesticides in vegetables and fruits by supercritical fluid extraction and liquid chromatography-tandem mass spectrometry. *Food Hygiene and Safety Science (Shokuhin Eiseigaku Zasshi)*, 55, 142-151.

26. Saleh, H., Elhenawee, M., Hussien, E.M., Ahmed, N., & Ibrahim, A.E. (2021). Validation of hplc-uv multi-residue method for the simultaneous determination of tetracycline, oxytetracycline, spiramycin and neospiramycin in raw milk. *Food Analytical Methods,* 14, 36-43.

27. Sánchez-Hernández, L., Hernández-Domínguez, D., Bernal, J., Neusüß, C., Martín, M.T., & Bernal, J.L. (2014). Capillary electrophoresis–mass spectrometry as a new approach to analyze neonicotinoid insecticides. *Journal of Chromatography A,* 1359, 317-324.

28. Tomlin, C.D. (2009). The pesticide manual: a world compendium. British Crop Production Council.

29. Valverde, S., Ares, A.M., Bernal, J.L., Nozal, M.J., & Bernal, J. (2018). Fast determination of neonicotinoid insecticides in beeswax by ultra-high performance liquid chromatography-tandem mass spectrometry using an enhanced matrix removal-lipid sorbent for clean-up. *Microchemical Journal,* 142, 70-77.

30. Wang, Y., Shen, L., Gong, Z., Pan, J., Zheng, X., & Xue, J. (2019). Analytical methods to analyze pesticides and herbicides. *Water Environment Research,* 91, 1009-1024.

31. Watanabe, E., Iwafune, T., Baba, K., & Kobara, Y. (2016). Organic solvent-saving sample preparation for systematic residue analysis of neonicotinoid insecticides in agricultural products using liquid chromatography− diode array detection. *Food Analytical Methods,* 9, 245-254.

32. Watanabe, E., Yamasaki, T., Hirakawa, Y., Harada, A., Iwasa, S., & Miyake, S. (2018). Organic solvent-free immunoassay for quantitative detection of neonicotinoid acetamiprid residues in agricultural products. *Analytical Methods,* 10, 3162-3169.

33. Xiao, Z., Li, X., Wang, X., Shen, J., & Ding, S. (2011). Determination of neonicotinoid insecticides residues in bovine tissues by pressurized solvent extraction and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B,* 879, 117-122.

34. Yan, S., Wang, J., Zhu, L., Chen, A., & Wang, J. (2015). Toxic effects of nitenpyram on antioxidant enzyme system and DNA in zebrafish (Danio rerio) livers. *Ecotoxicology and Environmental Safety,* 122, 54-60.

35. Yang, S.-Y., Koo, Y.-C., Wang, Z., Heo, K., Kim, H.-K., An, E.-M., Shin, H.-S., Lee, J.-W., & Lee, K.-W. (2010). Analysis of four pesticides, isoproturon, phenmedipham, pyridate and nitenpyram residues by high-performance liquid chromatography with diode-array detector. *Journal of Korean Society of Food Science and Nutrition,* 39, 1165-1170.

36. Yoshida, T., Murakawa, H., & Toda, K. (2013). Determination of nitenpyram and its metabolites in agricultural products by using hydrophilic interaction liquid chromatography-tandem mass spectrometry. *Journal of Pesticide Science,* 38, 27-32.

37. Zhang, M., Zhang, H., Zhai, X., Yang, X., Zhao, H., Wang, J., Dong, A., & Wang, Z. (2017). Application of β-cyclodextrin–reduced graphene oxide nanosheets for enhanced electrochemical sensing of the nitenpyram residue in real samples. *New Journal of Chemistry,* 41, 2169-2177.

38. Zhang, Q., Wang, X., Li, Z., Jin, H., Lu, Z., Yu, C., Huang, Y.-f., & Zhao, M. (2018). Simultaneous determination of nine neonicotinoids in human urine using isotope-dilution ultra-performance liquid chromatography–tandem mass spectrometry. *Environmental Pollution,* 240, 647-652.

39. Zhang, S., Yang, X., Yin, X., Wang, C., Wang, Z., 2012. Dispersive liquid–liquid microextraction combined with sweeping micellar electrokinetic chromatography for the determination of some neonicotinoid insecticides in cucumber samples. *Food Chemistry,* 133, 544-550.