

Determination of Neonicotinoid (Nitenpyram, Imidacloprid and Acetamiprid) Pesticides Residues in Five Dried Vegetables by High Performance Liquid Chromatography

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ABSTRACT

A method using dispersive solid-phase extraction (d-SPE) cleanup followed by QuEChERS with High Performance Liquid Chromatography (HPLC) has been established for determination of Neonicotinoid pesticide residues in various vegetables including brinjal, salad leaves, coriander, cabbage and lady finger. Based on an assessment of the HPLC characteristics, validation experiments are conducted for three Neonicotinoid pesticides i.e. Nitenpyram, Imidacloprid and Acetamiprid. In acidified acetonitrile analytical extraction is done with NaCl and MgSO₄ followed by activated neutral carbon. The method show good linearity ($R^2 \geq 0.999$) and relative standard deviation (RSD $\leq 3.36\%$). Average recoveries of three insecticides are in the range of 55% to 82% at spiking levels of 275.68 to 409.3 mg.Kg⁻¹. LOD and LOQ of three Neonicotinoid pesticides are in the range of 2.658 - 3.761 mg.Kg⁻¹ and 8.056 - 11.4 mg.Kg⁻¹. This QuEChERS determination method by HPLC is precise, simple, sensitive, accurate, easy to perform, and diversified at slightly higher concentrations. Moreover, it is very efficient and effective method and it can be utilized for regular monitoring of pesticide residues in drinking, canal and river water to save the life of organisms and to keep the aquatic environment clean.

Keywords: Neonicotinoids; Vegetables; High performance liquid chromatography; Limit of detection; Limit of quantification; Relative standard deviation

INTRODUCTION

Neonicotinoid are fast growing class of insecticides with a distinct mode of action. The commercially available Neonicotinoids are categorized into Acetamiprid, Imidacloprid, and Nitenpyram, which were previously introduced in Japan during 1990s [1]. Neonicotinoid have high affinity for insects and nicotinic acetylcholine receptors are widely used to protect crops against a broad range of pests, including aphids, whitefly, trips, and mealy bugs [2-4]. Their high polarity and other physicochemical properties make them useful for a wide range of applications such as seed treatment, soil drench, foliar and stem growth [5-7]. Before harvest, usually pesticides are applied daily to the crops. Therefore, the residual pesticides concentrations on crops immediately after harvest are relatively high. Sales of

Acetamiprid, Imidacloprid, and Nitenpyram were \$76.7 million in 1998 and accounted for 6.8% of the total insecticides distributed in Japan [8]. Recently, production of crops and applicability of different types of pesticides seem to be parallel. The pesticides should be used in limited extent for safe crops other food stuffs production. Excess amount of pesticide residues on food stuffs after their supplementary use is harmful for all living beings [9-11]. Since, these pesticides affect the functioning of living systems soon after their consumption as food [12,13]. Consequently, the European Community established maximum residue levels (MRLs) for each pesticide on food stuffs according to their toxicity level [14].

To examine the level of pesticides in foodstuffs, efficient analytical procedures are required. In this context, several

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Received: May 04, 2020; Accepted: May 18, 2020; Published: May 25, 2020

Citation: Sajid M, Razaq M, Ali Z, Hussain D, Hussain A, et al. (2019) Determination of Neonicotinoid (Nitenpyram, Imidacloprid and Acetamiprid) Pesticides Residues in Five Dried Vegetables by High Performance Liquid Chromatography. J Chromatogr Sep Tech. 11:431. DOI: 10.35248/2157-7064.20.11.431

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scientists developed analytical methods to quantify the amounts of these pesticides on crops [15,16]. Tsumura determined nitenpyram in food stuffs with an HPLC system [17] which involves silica gel column chromatography, diatomaceous earth column and acetone extraction. Nitenpyram was eluted in dichloromethane with diatomaceous earth column [18]. But dichloromethane was not found suitable for this method owing to environmental concerns [19]. Fernandez-Alba investigated imidacloprid in food stuffs by both HPLC-UV and LC-MS [20]. Imidacloprid was extracted by acetone and transferred in the mixture of petroleum ether and dichloromethane to avoid interferences. In vegetables, Navalon analyzed imidacloprid residues through hydrolysis method on GC-MS [21]. A Japanese official method is effectively used to determine pesticides on foods through GC-NPD [22]. Sasaki estimated polar compound Acetamiprid, via matrix-induced enhancement effect by GC [23]. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are useful for the analysis of Neonicotinoid in various matrices [9,24]. The simultaneous determination of multiple Neonicotinoids were examined by various methods including HPLC coupled with diode array or UV detection [24], LC-MS and tandem mass spectrometry (LC-MS/MS) [25,26].

The aim of current study is to develop a routine analysis method that preferably extends the current conventional multi-residue methods to integrate and quantify Neonicotinoid pesticides into the regular monitoring program. HPLC-UV is selected for the quantification of nitenpyram, acetamiprid and imidacloprid by QuEChERS [15,27].

SUBJECTS AND METHODS

Chemicals and reagents

Pesticide standards were purchased from **Lianyungang Liben Agro-Chemical Co., Ltd.** Magnesium sulfate anhydrous (MgSO₄) and sodium sulfate (Na₂SO₄) were purchased from MERCK EMSURE®. CAS 7487-88-9, EC Number 231-298-2, and MERCK EMSURE® ACS, ISO, Reag. Ph Eur. CAS No. 7757-82, respectively. Sodium chloride (anhydrous) was purchased from Sigma-Aldrich, beads, 1–10 mesh, 99.99% trace metals basis. Ultra-residue reagent acetonitrile (C₂H₃N) with ~100% purity and methanol (HPLC gradient grade) were taken from VWR Prolabo. Similarly, ultra-HPLC grade water was provided by Tedia. Vegetables (Lady Finger, Coriander, Cabbage, Brinjal and Salad leaves) were purchased from local market Multan, Pakistan.

Sample preparation

1000 ppm reference standard stock solution of each Neonicotinoid pesticide (Imidacloprid 95%, acetamiprid 95% and Nitenpyram 95%) was prepared. Standard multicomponent working solutions (10 ppm, 20 ppm, 30 ppm and 40 ppm) were prepared by diluting stock solution with mobile phase acetonitrile, methanol and water (20:20:60 v/v/v). These working solutions were used for spiking of samples and to draw the calibration curve. The vegetables i.e. Lady Finger, Coriander, Cabbage, Brinjal and Salad leaves samples were prepared with

the modified QUECHERS method. Blank sample of vegetables were prepared for fortification experiments and for the matrix-matched standards.

Initially, vegetables were washed thrice with distilled water to remove dust and insoluble particles and dried under sunlight for few days. Afterwards, they were ground into fine powder using a blender. The resulting powder was sieved with the help of sieve (12 mesh size). On the other side, Na₂SO₄ and MgSO₄ were dried in oven (Mammert, Universal Oven UN75) at 550 °C for 5 hours and stored in desiccator (Sci-Labware). Then 0.1 g of each dried ground vegetable sample was taken in centrifuge tube, soaked with 2 mL distilled water for 30 minutes. Afterwards, 7 mL of multicomponent working solutions were poured on top of them and vortex for 1 minute followed by addition of 7 mL acetonitrile with 1 minute vigorous shaking. These solutions were agitated for 30 minutes. Subsequently, 1.0 g NaCl and 4 g anhydrous MgSO₄ were added in each centrifuge tubes. All tubes were centrifuged in TGL-16 high speed centrifuge machine at 6000 rpm for 5 minutes. The separated acetonitrile layer was centrifuged for 5 minutes after adding 50 mg activated carbon and 150 mg MgSO₄. Finally acetonitrile layer was filtered through Whatman® filter paper 42 and analyzed by HPLC. The whole procedure is shown in Figure 1.

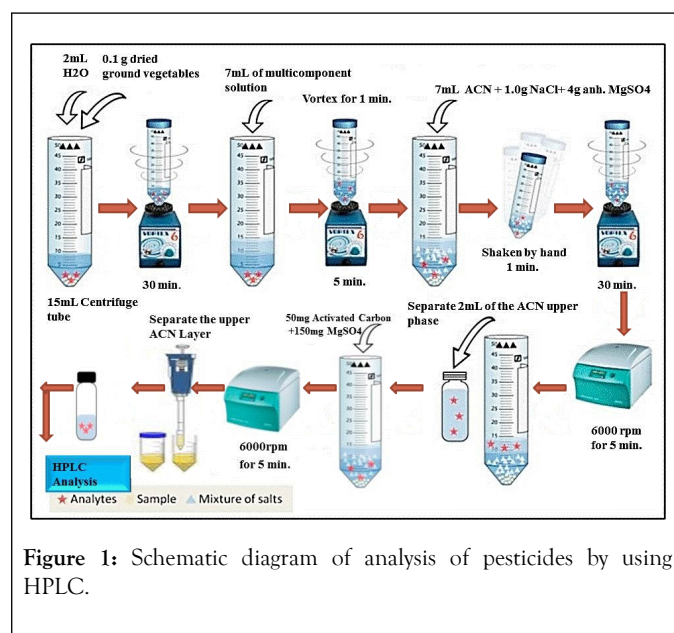


Figure 1: Schematic diagram of analysis of pesticides by using HPLC.

RESULTS AND DISCUSSION

Extraction and HPLC analysis of pesticides from vegetables samples

Neonicotinoid pesticides including Nitenpyram, Acetamiprid and Imidacloprid (Table 1) are investigated in five different types of vegetable samples including brinjal, salad leaves, coriander, cabbage and lady finger. Initially standard pesticide sample (20 ppm), blank samples, vegetable sample and spiked pesticide sample in relevant vegetable sample are analyzed through HPLC (LC-20AT Shimadzu Corporation, Japan) in cabbage (Fig. 2), salad leaves (Fig. 3), lady finger (Fig. 4), coriander (Fig. 5) and brinjal (Fig. 6). In blank, Nitenpyram is present in four

vegetables but absent in lady finger, while Acetamiprid is found in cabbage and in coriander and absent in other three vegetable

samples. Imidacloprid is the only pesticide which was not found in any vegetable sample during analysis of blank (Fig. 2a-6a).

Table 1: Chemical Structures of Three Neonicotinoid pesticides (5, 28).

No.	Compound	Chemical Structure	Water Solubility (g.L1)	Log Kw
1	Acetamiprid		4.25	0.80
2	Imidacloprid		0.61	0.57
3	Nitenpyram		≥59	-0.66

Then all three pesticide residues are extracted from spiked vegetable samples. Acetonitrile is selected as extracting solvent since Neonicotinoids pesticides are polar in nature with high water solubility. Obana used ethyl acetate as extracting solvent for Nitenpyram, Imidacloprid and Acetamiprid in cucumber, potato, tomato, eggplant, Japanese radish, and grape with 5%, 3% and 29% recoveries, respectively [28]. In another experiment, acetone extraction illustrates 0%, 24% and 25% recoveries for above mentioned insecticides [1]. By using the reference of these experiments, acetonitrile is selected as solvent for the extraction of Neonicotinoid pesticides. No pesticide residues are visible in HPLC analysis vegetable samples before spiking (Figure 2c-Figure 6c). But after extraction with acetonitrile, pesticide residues are particularly detected in spiked vegetables samples (Figures 2d-6d). The main difference in these methods is that the vegetable samples are soaked in water before using acetonitrile. The reason is that water is also hydrophilic and in combination with an organic solvent, it can significantly recover hydrophilic pesticides. So recoveries of Neonicotinoids are enhanced up to appreciable levels [29].

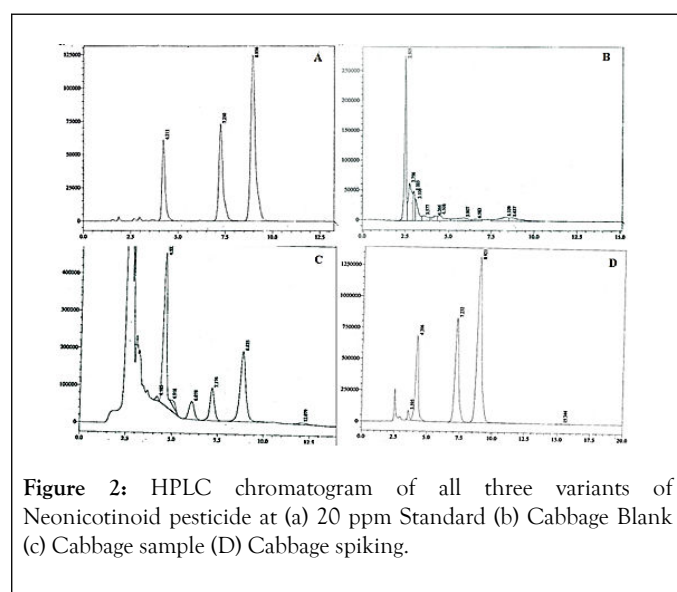


Figure 2: HPLC chromatogram of all three variants of Neonicotinoid pesticide at (a) 20 ppm Standard (b) Cabbage Blank (c) Cabbage sample (D) Cabbage spiking.

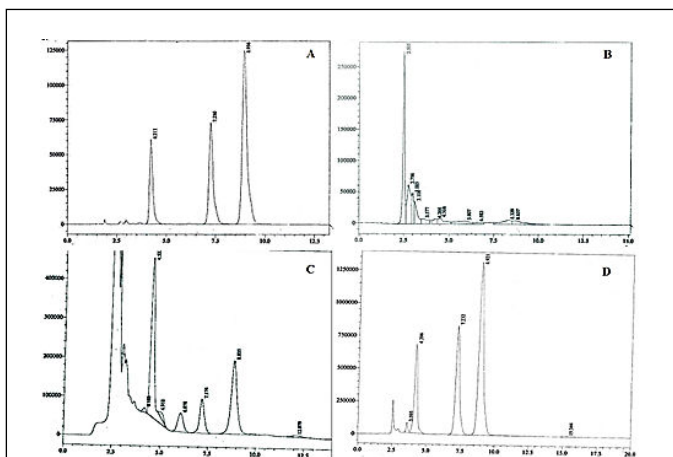


Figure 3: HPLC chromatogram of all three variants of Neonicotinoid pesticide at 20 ppm standard (b) Salad leaves Blank (c) Salad leaf sample (d) Salad leaf spiking.

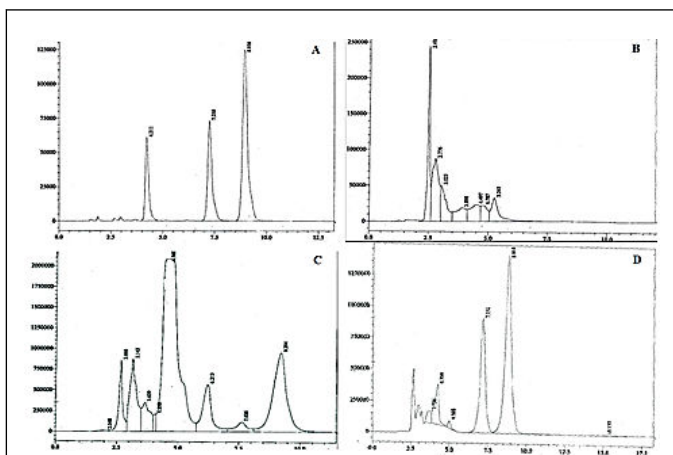


Figure 4: HPLC chromatogram of all three variants of Neonicotinoid pesticide at (a) 20 ppm standard (b) Lady finger Blank (c) Lady finger sample (d) Lady finger spiking.

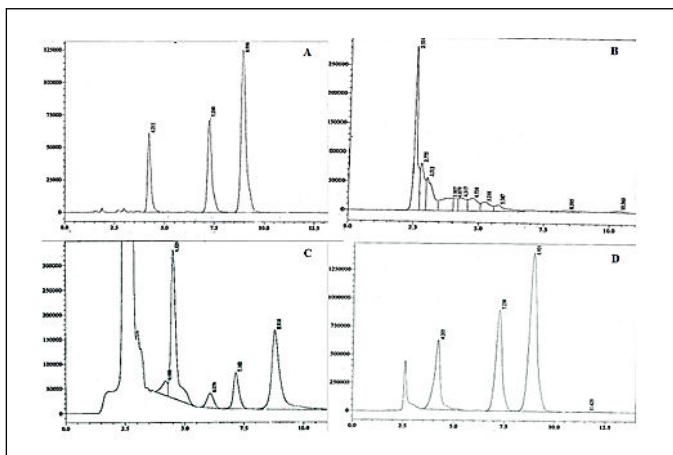


Figure 5: HPLC chromatogram of all three variants of Neonicotinoid pesticide at (a) 20 ppm standard (b) Corriander Blank (c) Corriander sample (d) Corriander spiking.

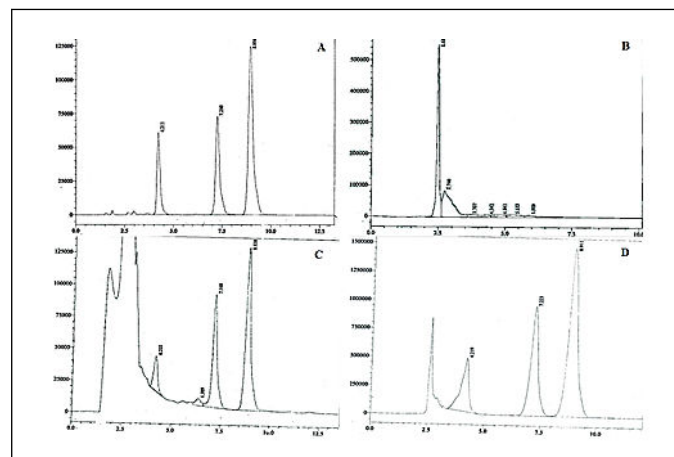


Figure 6: HPLC chromatogram of all three variants of Neonicotinoid pesticide at (a) 20 ppm standard (b) Bingil Blank (c) Bingil sample (d) Bingil spiking.

Calibration curve of standard solutions of each Neonicotinoid pesticide (Nitenpyram, Imidacloprid and Acetamiprid) is drawn with correlation coefficient (R^2) values of 0.9976, 0.9984 and 0.999, respectively (Fig. 7). The extracted amount of Acetamiprid, Nitenpyram and Imidacloprid in vegetable samples was calculated by plotting graphs of peak area versus concentration.

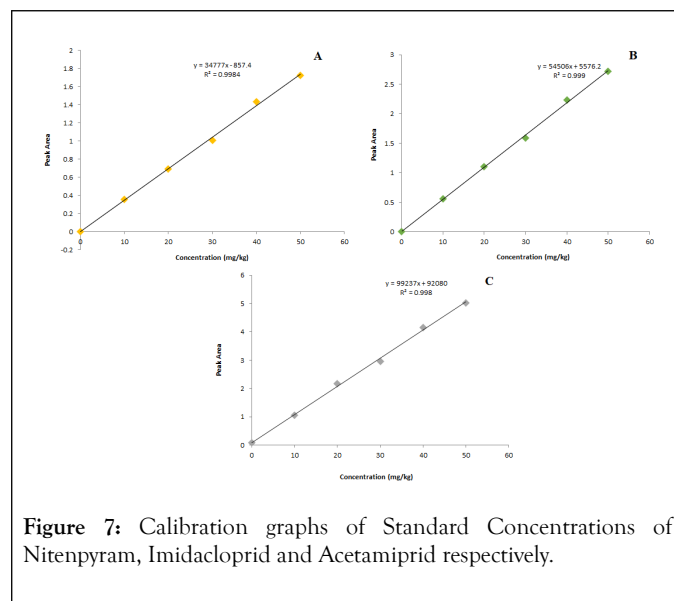


Figure 7: Calibration graphs of Standard Concentrations of Nitenpyram, Imidacloprid and Acetamiprid respectively.

Recovery analysis of pesticides

Nitenpyram, Imidacloprid and Acetamiprid are spiked at 2 levels i.e. 40 and 500 ppm in five vegetable samples as shown in Table 2. These insecticides are recovered in the range of 55-100% with 0.727-3.36% of relative standard deviation (RSD) at both spiking levels. There is no significant difference in recoveries among sample types or spiking levels.

The recovery of Nitenpyram in brinjal at 40 ppm spiking is 28% which is quite low. However, in salad leaves, coriander, cabbage and lady finger, the recovery of Nitenpyram is high i.e. 225%, 336%, 411% and 104%, respectively. The results indicate that these vegetables are positive with Nitenpyram. The recovery of

Imidacloprid in brinjal, salad leaves, coriander and cabbage is in between 55% and 72%, but its recovery for lady finger is 684%. In this way, the recoveries of Acetamaprid in brinjal, cabbage,

coriander, and lady finger are in the range of 58% to 100%. In contrast, its recovery for salad leaves is 122% that is indication of positive result.

Table 2: Recoveries of the Neonicotinoids at Spiking Levels 40,400 mg.Kg-1

Pesticide	Vegetables	Recovery mg.Kg-1	Recovery %	Recovery mg.Kg-1	Recovery %
		40mg.Kg-1		400mg.Kg-1	
Nitenpyram	Brinjal	11.01145	28	317.43	63
	Salad leaves	89.912	225	275.688	55
	Coriander	134.312	336	381.576	76
	Cabbage	164.345	411	290.5	58
	Lady finger	41.677	104	181.24	36
Imidacloprid	Brinjal	26.818	67	409.33	82
	Salad leaves	29.129	72	302.88	60
	Coriander	22.052	55	368.1005	73
	Cabbage	27.805	70	311.612	62
	Lady finger	273.77	684	395.839	79
Acetamaprid	Brinjal	23.389	58	405.318	81
	Salad leaves	48.966	122	304.295	61
	Coriander	40.171	100	368.744	74
	Cabbage	40.728	100	309.67	62
	Lady finger	38.745	97	397.3	78

Spiking at 400 ppm shows that the peak areas of each insecticide are enhanced to some extents. The recovery of Nitenpyram in brinjal is 371.430 mg.Kg-1 (63%) with peak area 11031976, recovery in salad leaves is 275.688 mg.Kg-1 (55%), recovery in coriander is 381.576 mg.Kg-1 (76%), recovery in cabbage is 290.5 mg.Kg-1 (58%) at the same retention time (Rt) 4.2 min, and the recovery in lady finger is 181.240 mg.Kg-1 (36%), that is low and it may due to the presence of some interferences. Recovery of imidacloprid in brinjal is 409.33 mg.Kg-1 (82%), recovery in salad leaves is 302.88 mg.Kg-1 (60%), recovery in coriander is 368.1005 mg.Kg-1 (73%), recovery in cabbage is 311.612 mg.Kg-1 (62%) and the recovery in lady finger is 395.839 mg.Kg-1 (79%) at the same retention time (Rt) 7.2 min. The recovery of Acetamaprid in brinjal, salad leaves, coriander, cabbage and in lady finger are in between 304.295 mg.Kg-1 to 405.318 mg.Kg-1 (61% to 81%) at the same retention time (Rt) 8.9 min. and 295 mg.Kg-1 to 405.318 mg.Kg-1 (61% to 81%) at the same retention time (Rt) 8.9 min.

Limit of detection and limit of quantitation

Optimized method is then applied to determine the limit of detection (LOD), limit of quantification (LOQ), relative standard deviation (RSD) and correlation coefficient (R²) of pesticides from vegetable samples (Table 3). R² values of all the pesticides are in the range of 0.99, RSD for Nitenpyram (3.36%), Imidacloprid (0.78%) and Acetamiprid (0.99%). Similarly, LOD and LOQ of all three pesticides range from 2.76 ng.mL⁻¹ to 3.3 ng.mL⁻¹ and 8.05 ng.mL⁻¹ to 11.4 ng.mL⁻¹, respectively.

Table 3: Relative Standard Deviation (RSD), Correlation Co-efficient (R²), Limit of Detection (LOD) and Limit of Quantitation (LOQ) of Neonicotinoid Pesticides.

Compound	R ²	RSD	LOD	LOQ
Nitenpyram	0.9984	3.36%	3.322	10.066
Imidacloprid	0.999	0.73%	2.658	8.056

Acetamiprid	0.998	1.00%	2.761	11.4
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Determination of neonicotinoid concentration in vegetable samples

Concentration of all three residues of Neonicotinoid is also determined from non-spiked vegetable sample. Results demonstrate that highest concentration of Nitenpyram is present in four vegetables samples including Brinjal, Cabbage, Salad Leaves and Coriander while Imidacloprid is not found in any of five vegetables (Table 4). On the other hand, no pesticide is detected in lady finger samples.

Table 4: Neonicotinoid concentration (mg. Kg⁻¹) in blank samples of vegetables.

Samples	Imidacloprid	Acetamiprid	Nitenpyram
Brinjal	-----	-----	4.162
Cabbage	-----	2.423	3.84
Salad Leaves	-----	-----	12.605
Coriander	-----	1.033	13.111
Lady finger	-----	-----	-----

CONCLUSION

QuEChERS method depends on the dSPE cleanup process in which MgSO₄, NaCl and activated carbon are utilized. The recoveries of three Neonicotinoid pesticides in vegetable samples are good to some extents. Average recoveries of Neonicotinoid were ranged from 55% to 82% at spiking levels with relative standard deviation (RSD) was from 0.7278% to 3.36%. LOD of three Neonicotinoids were in the range of 2.658 mg. Kg⁻¹ to 3.761 mg. Kg⁻¹ and LOQ of these Neonicotinoids were in the range of 8.055 mg. Kg⁻¹ to 11.397 mg. Kg⁻¹. In conclusion, QuEChERS - HPLC method is very rapid, effective, sensitive and simple method for determination of Neonicotinoid pesticide residues at slightly higher concentration in brinjal, salad leaves, coriander, and cabbage and lady finger, which can be use for regular monitoring of pesticide residues in drinking, canal and river water to save the life of organisms and to keep the aquatic environment clean.

ACKNOWLEDGMENT

Authors acknowledge Higher Education Commission (HEC) of Pakistan for the support in execution of this research. We also thanks Solex Chemicals, Industrial Estate (Pvt.) Ltd Multan, for their cooperation during the analysis of pesticides.

COMPLIANCE WITH ETHICAL STANDARDS

Authors declare no conflict of interest in this study. All the authors contributed equally to this research. The corresponding author designed the study and revised the manuscript. The manuscript is submitted with the approved consent of all authors.

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