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DETECTION OF THIAMETHOXAM, FLUBENDIAMIDE AND ENDOSULPHAN RESIDUES IN RICE GRAINS

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ABSTRACT

A method was standardized and developed for simultaneous detection of thiamethoxam, flubendiamide and endosulphan residues in rice grains. Detection of monocrotophos, deltamethrin, phosphamidon and dichlorvos was already developed in rice grains with the same instrumentation system. However, in this new methodology, extraction was done with acetonitrile and simultaneous liquid-liquid partitioning formed by adding anhydrous magnesium sulphate followed by a simple clean-up step known as dispersive solid-phase extraction. The final extracts were concentrated and made up the volume with HPLC grade acetonitrile. Detection of thiamethoxam, flubendiamide and endosulphan residues were estimated by using reverse phase-high performance liquid chromatography (RP-HPLC) coupled with UV-VIS detection system and C_{18} column. The mobile phase of acetonitrile and water (90:10, v/v) @ 0.5 ml/min flow rate was operated for the method with a retention time of 5 mins. Thiamethoxam, flubendiamide and α-endosulphan and β- endosulphan were found to get the peaks at retention times of 1.95 min, 2.40 min, 3.39 min, and 3.90 min respectively. The recovery percentage of above 80 was observed for all the three insecticides when samples were spiked with standard insecticides at different fortification levels of 0.05, 0.10, 0.25, 0.50 and 1.00 mg/kg.

Key words: Thiamethoxam, flubendiamide, endosulphan, residues, rice grains, detection, methodology, HPLC, acetonitrile, liquid- liquid partitioning

Rice (*Oryza sativa* L.) cultivation requires good monsoon or good irrigation facilities with hot and humid climate (Barrion et al., 2007). During its crop growth many biotic and biotic factors are affecting the crop (Behura et al., 2011). Major biotic factors that influence rice production in the northeastern hills are diseases, insect pests, rodents and weeds. Various insect pests are known to attack rice crop of which 20 species are considered important (Arora and Dhaliwal, 1996), and some are major (Jenita et al., 2016). Farmers rely on insecticides to combat the damage caused by insect pests, and insecticides like monocrotophos, dichlorvos, malathion, chloropyriphos, cypermethrin, deltamethrin, thiamethoxam, imidacloprid, carbofuran, chlorantraniliprole, flubendiamide, fipronil, etc. are used (Akoijam et al., 2019). Once the pesticides are applied without the recommended practices, these can harm biota and the surrounding environment (Aktar et al., 2009).

Information is very much limited about the safety of various insecticides in rice. Methods for the detection and determination of monocrotophos, deltamethrin, phosphamidon and dichlorvos residues in rice grains samples had been standardized by RP-HPLC equipped with UV-VIS detector (Akoijam et al., 2019). The methodology for the determination of thiamethoxam, flubendiamide and endosulphan residues is required for the detection of residues in rice samples. The present study is a method that is modified and based on work done and introduced by Anastassiades and Lehotay (2003). The method provides high recoveries for a wide polarity and volatility range of pesticides; very precise and concrete results are achieved due to an internal standard is used; very little space, glassware, and chemicals are used to carry out the extraction process.

MATERIALS AND METHODS

The technical grade analytical standards such as thiamethoxam (98.0%), flubendiamide (98.3%), endosulphan (99.4%) and primary secondary amine (PSA) sorbent were procured from sigma-aldrich, Kolkatta. Chemicals such as Sodium chloride, activated anhydrous magnesium sulphate ($MgSO_4$), anhydrous sodium sulfate and solvents like high-performance liquid chromatography (HPLC) grade acetonitrile, HPLC grade water was obtained from E. Merck

(India) Ltd, Mumbai, India. All the solvents used were of laboratory grade and were redistilled in all-glass apparatus before the experiment. The suitability of the solvents and other chemicals was ensured by running reagent blanks before real analysis.

The high-performance liquid chromatograph (series 200) was equipped with a reverse-phase, RP, C18 column and a UV-VIS detector, and dual pumps supplied by M/S Perkin Elmer, United States. The HPLC column, a Brownlee Analytical C18 column (150 mm column length, 4.6 mm inside diameter and 5 µm particle size) was also procured from M/S Perkin Elmer. For the control of the instrument, data acquisition, and processing, TC Nav software was used. A good satisfactory separation of peak symmetry was obtained with a gradient flow of acetonitrile (100 %) at a flow rate of 0.7 mL/min. Quantification was achieved with UV-VIS detection at 230 nm based on peak area with a retention factor of 10 min and an injection volume of 20 µL. Preparation of standard stock solution of thiamethoxam, flubendiamide, and endosulphan, construction of a calibration curve as well as the method of rice grains sample collection is same as that of the methods followed in Akoijam et al. (2019). The methodology for the extraction and clean-up process is done by modified QuEChERS (Akoijam et al., 2019).

RESULTS AND DISCUSSION

Reversed-phase HPLC, with UV-VIS detection, was shown to be good for the determination of thiamethoxam, flubendiamide and endosulphan residues because of no need for derivatization step. Chromatographic separation in C18 columns provides a good outcome. The detection at 225 nm provides suitable chromatograms for the quantification of thiamethoxam, flubendiamide and endosulphan residues in real samples. Under the preferred conditions, thiamethoxam, flubendiamide and α-endosulphan and β- endosulphan showed retention factors of 1.95 min, 2.40 min, 3.39 min and 3.90 min respectively (Fig.1). The fortified samples 0.05, 0.10, 0.25, 0.50 and 1.00 mg/kg levels of thiamethoxam, flubendiamide, α-endosulphan and β- endosulphan were found to be in the range of 82.18 to 88.34 %, 80.90 to 91.95 %, 82.24 to 98.27 % and 86.71 to 97.80 % respectively (Table). The same instrumentation system coupled with modified QuEChERS methodology has also developed the method for the determination of different insecticides viz. monocrotophos and deltamethrin, and phosphamidon and dichlorvos residues at different detection wavelengths of 225 nm and 210 nm respectively. Under these conditions, monocrotophos and deltamethrin depicted at retention times at 0.77 min and 3.01 min and that of phosphamidon and dichlorvos residues were found at different peaks at 1.11 min and 1.39 mins respectively (Akoijam et al., 2019). However, al the methods including the present finding method, the run time for detecting the residues is up to 5 mins.

The specific, simplified, quick, cost-effective RP-HPLC method by using QuEChERS extraction method can be used for the determination of different groups of insecticides like thiamethoxam, flubendiamide and endosulphan residues from rice grains sample by following the described instrumentation method by

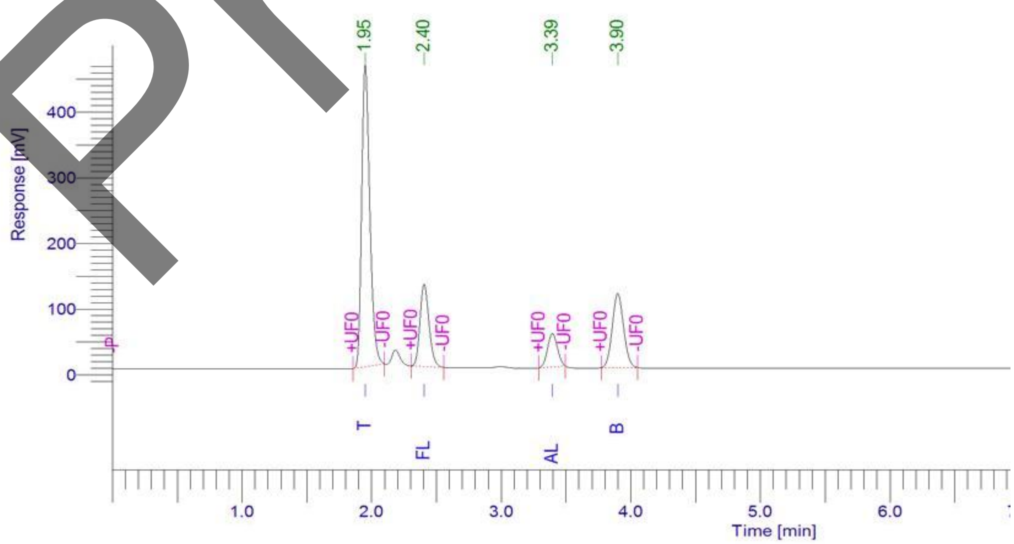

Fig. 1. HPLC chromatograms of standards of thiamethoxam, flubendiamide, α-endosulphan and β- endosulphan in which retention times on x-axis and % deflection on y-axis.

Table 1. Recovery (%) of thiamethoxam, flubendiamide, α-endosulphan and β- endosulphan rice grains (n=5)

Level of fortification (mg kg^{-1})	Thiamethoxam	Flubendiamide	Alpha Endosulphan	Beta-endosulphan
0.05	82.56±2.08	89.09±0.67	83.09±2.56	91.08±0.98
0.10	84.24±1.67	91.95±1.80	98.12±1.21	86.71±0.01
0.25	88.34±0.98	83.90±1.45	88.67±1.89	95.74±1.05
0.50	82.18±2.87	82.89±2.43	98.27±2.51	88.98±1.78
1.00	84.34±0.67	80.90±3.67	82.24±3.00	97.80±1.56

[a]Mean ± Standard deviation

following the extraction and clean-up procedure. The consistent recoveries ranging from 82.18 to 98.27 % for thiamethoxam, flubendiamide and α-endosulphan and β-endosulphan were observed when samples were spiked at different levels of 0.05, 0.10, 0.25, 0.50 and 1.00 mg/kg levels. The present QuEChERS methods are more effective and provide the quickest, an easy and cheap method as compared to that of the traditional methods.

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